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THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Regular physical activity affects all tissues in the human body, not least skeletal muscle. The skeletal muscle adaptation involves changes that help the muscle to handle perturbations of the intracellular homeostasis during exercise and thereby gradually improve the exercise capacity. Skeletal muscle has a remarkable ability to dynamically remodel and its adaptive process depends on *e.g.* exercise intensity and the training status of the individual performing the exercise. An important aerobic adaptive process is mitochondrial biogenesis, a process regulated by a complex network of transcriptional regulators inside the muscle cell. Using three different exercise interventions in humans, acute aerobic- and resistance exercise, and three weeks of HIIT, and obtaining skeletal muscle biopsies at various time points, the overall aim of this thesis was to expand the knowledge of molecular mechanisms involved in human skeletal muscle adaptation to exercise and exercise training, with a special emphasis on mitochondrial biogenesis. With three weeks of HIIT there was an overall attenuation of the transcriptional response to a single exercise bout. However, the response pattern was strikingly reproducible and independent of training status since the vast majority of differentially expressed genes at the last exercise bout were regulated at the first bout, albeit to a lesser degree. Additionally, there were different genes and pathways responding to acute exercise compared to training. At least four variants of the 'master regulator' of mitochondrial biogenesis, PGC-1α, were present in human skeletal muscle. All PGC-1α variants were increased by aerobic- and resistance exercise and there was no support for an exercise-modality specific variant. Moreover, PGC-1α-ex1b protein was induced earlier than PGC-1α protein after acute aerobic exercise. The transcriptional repressors RIP140 and p107 were affected by acute exercise and training, respectively. In conclusion, the acute transcriptional response in skeletal muscle after exercise is blunted as the muscle adapts to training, supporting the fundamental concept that 'trained' muscle can maintain the cellular homeostasis better than 'untrained' muscle and thereby is exposed to a lower stimulus during acute exercise. Also, it seems like there are generally other genes orchestrating the remodelling process than those coordinating the maintenance of skeletal muscle adaptation. The control of both hypertrophy and aerobic adaptations are most likely coordinated through a much broader array of transcription factors, and other molecular mechanisms, than a single coactivator of transcription. Also, more emphasis should be put on the importance of transcriptional repressors in the regulation of skeletal muscle adaptation to exercise training.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals:

I. **Ydfors M**, Fischer H, Mascher H, Blomstrand E, Norrbom J*, Gustafsson T*. The truncated splice variants, NT-PGC-1α and PGC-1α4, increase with both endurance and resistance exercise in human skeletal muscle. *Physiol Rep.* 2013 Nov;1(6):e00140.

II. Gidlund E-K, **Ydfors M**, Appel S, Rundqvist H, Sundberg CJ, Norrbom J. Rapidly elevated levels of PGC-1α-b protein in human skeletal muscle after exercise: exploring regulatory factors in a randomized controlled trial. *J Appl Physiol.* 2015 Aug 15;119(4):374–84.

III. Bhattacharya D*, **Ydfors M***, Hughes MC, Norrbom J, Perry CGR, Scimè A. Decreased transcriptional corepressor p107 is associated with exercise-induced mitochondrial biogenesis in human skeletal muscle. *Physiol Rep.* 2017 Mar;5(5):e13155.

IV. **Ydfors M**, Sundberg CJ, Perry CGR, Rullman E*, Norrbom J*. Human skeletal muscle transcriptome in response to high-intensity interval training. *In manuscript.*

* These authors contributed equally.

Publication by the author not included in the thesis:

Ydfors M, Hughes MC, Laham R, Schlattner U, Norrbom J, Perry CGR. Modelling in vivo creatine/phosphocreatine in vitro reveals divergent adaptations in human muscle mitochondrial respiratory control by ADP after acute and chronic exercise. *J Physiol*. 2016 Jun 1;594(11):3127–40.

CONTENTS

LIST OF ABBREVIATIONS

1RM	One-repetition maximum
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CaMK	Ca^{2+}/calmodulin-dependent kinases
cDNA	Complementary deoxyribonucleic acid
DNA	Deoxyribonucleic acid
ERRα	Estrogen-related receptor alpha
ERRγ	Estrogen-related receptor gamma
FDR	False discovery rate
GSEA	Gene-set enrichment analysis
HIIT	High-intensity interval training
HR_{max}	Maximal heart rate
IQR	Interquartile range
LMM	Linear mixed models
LSD	Fisher's least significant difference
MAPK	Mitogen-activated protein kinases
mRNA	Messenger ribonucleic acid
mtDNA	Mitochondrial DNA
mTOR	Mammalian target of rapamycin
NCoR1	Nuclear receptor corepressor 1
NRF	Nuclear respiratory factor
NT-PGC-1α	N-truncated peroxisome proliferator-activated receptor gamma coactivator 1-alpha

OXPHOS	Oxidative phosphorylation
p107	Retinoblastoma-like 1
p53	Tumor protein p53
PCR	Polymerase Chain Reaction
PGC	Peroxisome proliferator-activated receptor gamma coactivator
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPARγ	Peroxisome proliferator-activated receptor gamma coactivator
Q	Quartile
Rb	Retinoblastoma protein
RIP140	Nuclear receptor interacting protein-1
rpm	Revolutions per minute
RT-PCR	Reverse Transcriptase PCR
SD	Standard deviation
SDH	Succinate dehydrogenase
SEM	Standard error of the mean
TFAM	Mitochondrial transcription factor A
TFB1M	Mitochondrial transcription factor B1
TFB2M	Mitochondrial transcription factor B2
VEGF	Vascular endothelial growth factor
VO_2max	Maximal oxygen uptake
VO_2peak	Peak oxygen uptake

1 BACKGROUND

Regular physical activity affects many tissues and organs of the human body and has many well-documented positive effects on health and performance (Gustafsson & Kraus 2001; Holloszy 1967; Gabriel & Zierath 2017; Haskell et al. 2007). The skeletal muscle tissue is highly affected by exercise and has a remarkable ability to dynamically remodel. The adaptations to exercise involve changes that help the skeletal muscle to handle the perturbations of the intracellular homeostasis during exercise and thereby gradually improve the exercise capacity.

1.1 SKELETAL MUSCLE TISSUE STRUCTURE AND FUNCTION

The skeletal muscle tissue (Figure 1) is the largest organ in the body and constitutes about 40-50 % of the total body mass. The larger part of the muscle tissue consists of long multi-nucleated tubular muscle fibres organized into fibre bundles surrounded by connective tissue (fascia). In addition to muscle fibres, the skeletal muscle tissue includes *e.g.* satellite cells important for the maintenance of the muscle fibres, nerve fibres and capillaries.

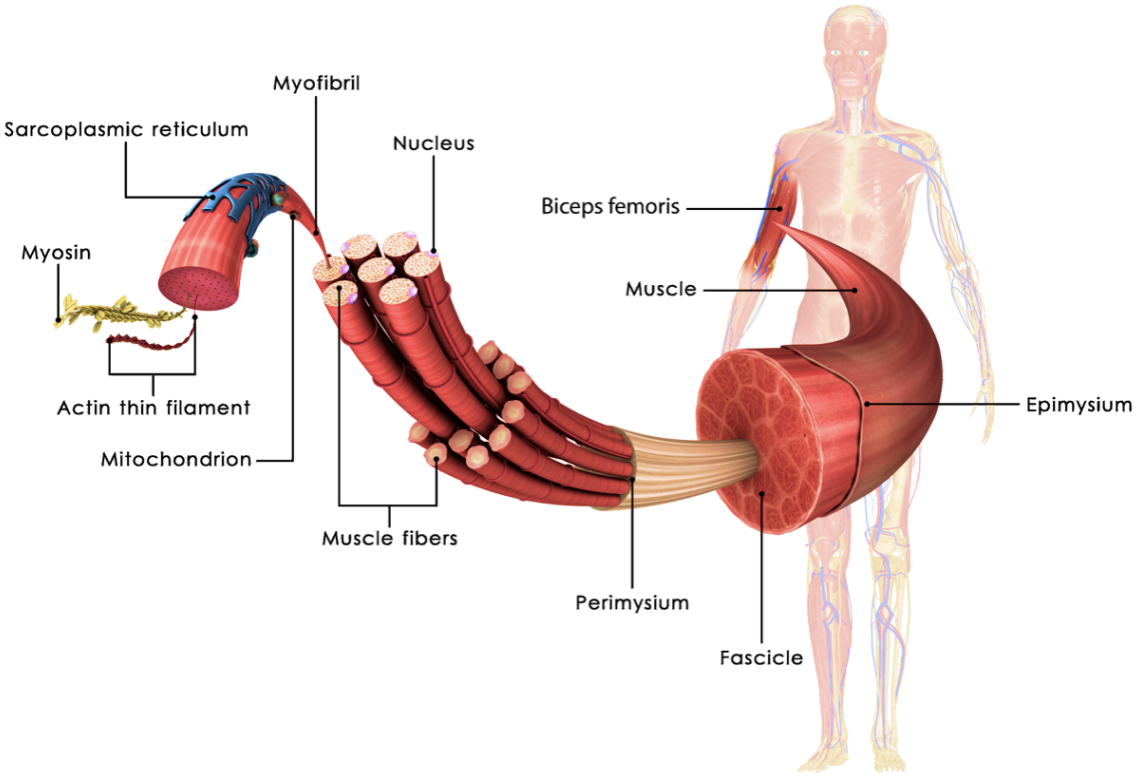

Figure 1. Illustration of skeletal muscle tissue. The skeletal muscle tissue consists of muscle fibres enclosed by the cell membrane, sarcolemma. Each muscle fibre contains myofibrils consisting of the contractile proteins myosin (yellow) and actin (red), as well as mitochondria (green), nucleus (purple), and Ca^{2+}-containing sarcoplasmic reticulum (blue). The muscle fibres are encircled by a network of capillaries (red) and organised into fascicles (bundles) surrounded by connective tissue, perimysium. These fascicles form the muscle that in turn is surrounded by the fascia, epimysium.

Each muscle fibre constitutes numerous myofibrils that are composed by repeated sections of sarcomeres. The sarcomeres are the smallest contractile units of the skeletal muscle. They are composed of *e.g.* the fibrous proteins actin and myosin (myofilaments) that slide against each other, leading to a shortening of the sarcomere thus creating the mechanical force that contracts the muscle. The action of the myofilaments, through cross-bridge cycling, is energy dependent and needs the high-energy molecule adenosine triphosphate (ATP).

1.2 SKELETAL MUSCLE ADAPTATION TO EXERCISE

Skeletal muscle tissue has a remarkable ability to dynamically remodel and adapt to the altered demands it is subjected to. Exercise training leads to pronounced, yet reversible, phenotypic changes of the skeletal muscle tissue and the cardiovascular system. The skeletal muscle adaptations to exercise training are the accumulated result of repeated bouts of acute exercise (Holloszy & Coyle 1984; Perry et al. 2010; Egan & Zierath 2013). The adaptations of the skeletal muscle to exercise training involves changes that help the muscle to handle the disturbance of the intracellular homeostasis during exercise and thereby gradually improve the exercise capacity.

The adaptive processes following regular exercise training depend on *e.g.* the type of training performed, the exercise frequency, duration and intensity, as well as the training status and the genetics of the individual performing the exercise (Roth et al. 2002; Robinson et al. 2017; Laursen & Jenkins 2002; MacInnis & Gibala 2017; Timmons et al. 2010). Different types of training, ranging from heavy resistance exercise to aerobic exercise, give rise to distinctive physiological effects. Each mode of exercise entails specific changes, ultimately leading to a phenotype somewhere in the adaptation continuum. Classical end-point adaptations to aerobic- and resistance exercise training includes improved endurance capacity and force production, respectively.

1.2.1 Aerobic exercise

Endurance exercise is characterised by low-resistance and high-repetition muscle actions with the purpose to improve the aerobic metabolic system and sustain a high-power output during an extended period of time. During aerobic exercise the energy demand in the muscle can increase by >100-fold making the energy systems and metabolism in the muscle important (Gaitanos et al. 1993). Aerobic exercise training results in *e.g.* an increase in mitochondrial density and function, increased metabolic enzyme activity, increased skeletal

muscle capillary density through angiogenesis, increased glycogen storage and utilization, and increased intracellular lipid stores, in the skeletal muscle tissue all contributing to improved aerobic capacity and resistance to fatigue (Drake et al. 2016; Spina et al. 1996; Gustafsson 2011; Andersen & J. Henriksson 1977; Booth & Thomason 1991; Holloszy 1975).

Contracting muscle use various energy sources, both intra- and extramuscular substrates, dependent on the duration and intensity of the exercise, but also on the diet, genetic factors, and training status of the individual. During short intense exercise the energy comes from stored intramuscular ATP and creatine phosphate (chemical energy is transferred from creatine phosphate to form new ATP from adenosine diphosphate (ADP)). During intense work up to 90 seconds, anaerobic lactate formation provides most of the ATP. During longer work, the ATP is formed through nutrient- (glucose and fatty acids) and oxygen-dependent oxidative phosphorylation (OXPHOS) in the inner mitochondrial membrane (Hargreaves 2000).

Maximal oxygen uptake (VO_2max), commonly referred to as maximal aerobic capacity or cardiorespiratory fitness, is the body's upper limit for consuming, distributing, and utilising oxygen for energy conversion, and is a good predictor of exercise performance. Aerobic exercise training leads to increased VO_2max through multiple central and peripheral adaptations with increased cardiac output being the main contributor and also the limiting factor for improvement of VO_2max (Bassett & Howley 2000). However, the metabolic and structural adaptations in the skeletal muscle tissue seen with aerobic exercise training is critical for improving submaximal performance. The peripheral adaptations include for example increased activity of mitochondrial enzymes enhancing substrate oxidation during submaximal exercise and increased capillarisation facilitating delivery of energy substrates and oxygen to the muscle tissue. Further evidence for that mitochondrial density and function are important for endurance capacity is that VO_2max has been correlated with mitochondrial content and respiratory function as well as lactate threshold and performance (Ivy et al. 1980; Jacobs et al. 2011; van der Zwaard et al. 2016; Hoppeler et al. 1973; Granata et al. 2016).

1.2.2 Mitochondria

The mitochondria are double membrane-enclosed organelles remarkably abundant in skeletal muscle cells. The mitochondria form a reticular network providing the cell with energy, distributed across the whole cell (Ogata & Yamasaki 1997; Glancy et al. 2015;

Picard et al. 2013). They are essential organelles as they, among other things, provide the cell with large amounts of the high-energy compound ATP, enabling energy demanding cellular processes. Hence, they are the primary controllers of cellular aerobic metabolism. Mitochondria are unique organelles since they contain their own circular deoxyribonucleic acid (DNA; mitochondrial DNA, mtDNA) that encodes for 37 proteins, of which 13 proteins are involved in the respiratory chain. The mitochondrial proteome consists of around 1,000 proteins with the majority being encoded by genes in the nuclear DNA (Taanman 1999; Mercer et al. 2011).

The mitochondrial reticulum is dynamic and undergoes remodelling through fusion, fission, and synthesis (Ryan & Hoogenraad 2007; Drake et al. 2016). Also, it is degraded through mitophagy to remove and degrade damaged components (Campello et al. 2014; Yan et al. 2012). The demands during physical activity, particularly aerobic exercise, that are put on the skeletal muscle tissue include its ability to extract energy, in the form of ATP, from nutrients such as lipids and carbohydrates. This process takes place in the mitochondria inside the cell. One way for the muscle cells to adapt to the increased energy demands with physical activity is to increase the mitochondrial mass, through mitochondrial biogenesis, which is a key process in skeletal muscle adaptation to aerobic exercise. Increased mitochondrial density is reflected by increased amounts and activity of mitochondrial enzymes in the TCA cycle, respiratory chain and fatty acid oxidation (Booth & Thomason 1991). Hence, increased mitochondrial mass lowers the disruptions of cellular homeostasis that occur during acute exercise. The morphology and function of the mitochondria are regulated through a complex network of transcriptional regulators controlling nuclear genes encoding mitochondrial proteins. These proteins include factors regulating the transcription and replication of the mitochondrial genome (Spiegelman & Heinrich 2004).

1.2.3 Resistance exercise

Resistance exercise, low-volume and high-force muscle actions, leads to skeletal muscle hypertrophy and increased strength and power (Narici et al. 1989; Tesch 1988). These adaptations are largely dependent on protein synthesis in the skeletal muscle fibres. Skeletal muscle hypertrophy predominantly occurs through increased protein content and cross-sectional area of the individual muscle fibres (Lüthi et al. 1986). Myonuclear addition mediated by satellite cells, to uphold the myonuclei- to cytoplasmic ratio and thereby maintain the transcriptional capacity of the muscle fibre, is another adaptation during muscle hypertrophy with resistance exercise training (Nielsen et al. 2012; Allen et al.

1999). The skeletal muscle adaptations to resistance exercise are regulated partly by other signalling pathways than those important for aerobic exercise adaptations (Egerman & Glass 2014).

1.2.4 High-intensity interval training

One of the major goals with aerobic exercise training is to improve cardiorespiratory fitness, as well as metabolic- and skeletal muscle function. High-intensity interval training (HIIT) is usually referred to as exercise at "near maximal" efforts with intensities that elicit more that 80 % of maximal heart rate (HR_{max}). HIIT has been shown to generate similar, or even greater, molecular changes in skeletal muscle as well as improvement in VO_2max, as that of conventional constant-load endurance-type training (Little et al. 2011; MacInnis & Gibala 2017; Gibala et al. 2012; Little et al. 2010; Tabata et al. 1996; Daussin et al. 2008; Milanović, Sporiš & Weston 2015a; Bacon et al. 2013; Helgerud et al. 2007; Wisløff et al. 2009; Bishop et al. 2019).

1.3 MOLECULAR REGULATION OF SKELETAL MUSCLE ADAPTATION

Each mode of acute exercise entails specific changes of the internal environment of the skeletal muscle tissue. To which degree it is affected depends on, as mentioned earlier, the exercise intensity and duration, genetics, as well as training- and dietary status of the individual performing the exercise. During the recovery after acute exercise the skeletal muscle tissue restores the cellular homeostasis and in response to repeated exercise bouts adapts to better cope with the next exercise bout.

The disruptions of cellular homeostasis and the various stimuli put on the skeletal muscle tissue during acute exercise includes, but are not limited to, mechanical load, ATP consumption with a subsequent shift in ADP/ATP ratio, increased adenosine monophosphate (AMP), reduces creatine phosphate and glycogen levels, increased reactive oxygen species, increased blood flow, drop in pH, metabolic perturbation, an increase in intracellular Ca^{2+} levels, and reduced oxygen tension, and leads to specific adaptations (Norrbom et al. 2004; Hawley et al. 2014), Coffey:2007kr}. These primary stressors activate several key signalling pathways important for the remodelling of the skeletal muscle tissue through *e.g.* AMP-activated protein kinase (AMPK), mitogen-activated protein kinases (ERK1/2, p38 MAPK), Ca^{2+}/calmodulin-dependent kinases (CaMK), calcineurin, and mammalian target of rapamycin (mTOR). Exercise affects the expression, and the enzyme activity, of these factors, leading to the activation of downstream targets

via *e.g.* phosphorylation and deacetylation. Subsequently, genes important for skeletal muscle remodelling are transcribed, including various transcriptional regulators that orchestrate the adaptive response (Chin 2005; Tavi & Westerblad 2011; Rose & Hargreaves 2003; Winder & Hardie 1996; Fujii et al. 2000; Widegren et al. 1998; McGee & Hargreaves 2010; Watson & Baar 2014; Andersen & J. Henriksson 1977).

1.3.1 Transcriptional regulators

Gene transcription is regulated through various transcriptional activators and repressors, as well as proteins binding to these transcription factors altering their function, *i.e.* transcriptional coactivators and -corepressors (Spiegelman & Heinrich 2004; Schnyder et al. 2017). Transcriptional regulators in relation to exercise have been studied comprehensively. Still, there are much that needs to be elucidated, especially the temporal changes in transcription of these regulators during the recovery period after acute exercise.

Peroxisome proliferator-activated receptor gamma (PPARγ) coactivators (PGCs), a family of transcriptional coactivators, coordinate the expression of many transcription factors important for mitochondrial biogenesis and oxidative metabolism. The transcriptional coactivator PGC-1 alpha (PGC-1α) has been recognised for many years as a ‘master regulator’ of mitochondrial biogenesis in skeletal muscle. Increased skeletal muscle transcription and protein expression of this ‘master regulator’ after exercise and exercise training is evident in numerous studies (Norrbom et al. 2004; Perry et al. 2010; Pilegaard et al. 2003; Russell et al. 2003; Short et al. 2003).

PGC-1α was first discovered as an interacting partner of the nuclear receptor PPARγ involved in thermogenesis in brown adipose tissue (Puigserver et al. 1998). PGC-1α is highly induced in skeletal muscle by exercise and interacts with a wide variety of transcription factors. Exercise activate kinases (*e.g.* AMPK and p38 MAPK) leading to phosphorylation, and subsequent activation, of PGC-1α (Jäger et al. 2007; Bishop et al. 2019). Upon activation, PGC-1α is translocated from the cytosol to the nucleus and mitochondria where it coactivates transcription (Safdar et al. 2011; Wright et al. 2007; Little et al. 2011). Post-translational phosphorylation also increase PGC-1α protein stability, which prolong its half-life leading to increased steady-state levels (Puigserver et al. 2001; Perry et al. 2010; Knutti et al. 2001; Fan et al. 2004), see Figure 2 for a schematic illustration.

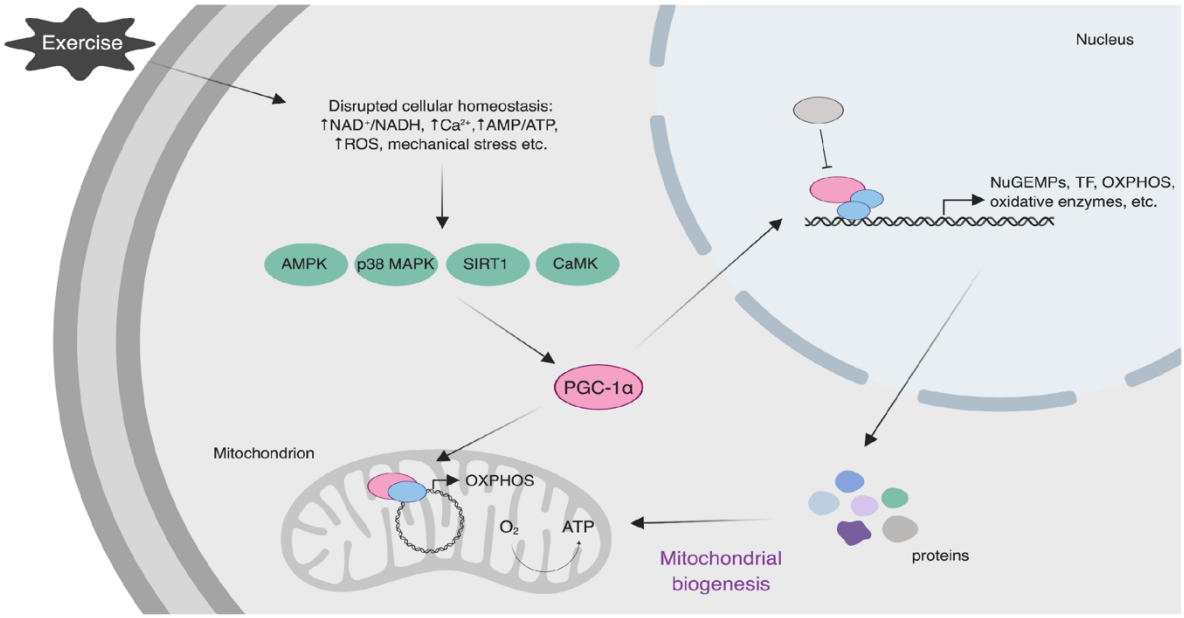

Figure 2. Schematic overview of molecular pathways in skeletal muscle after exercise and exercise training. Exercise leads to disrupted cellular homeostasis that initiate activation of various pathways and subsequent gene expression of *e.g.* nuclear genes encoding mitochondrial proteins (NuGEMPs) as well as transcription factors (TF) and coregulators. Translated TF and transcriptional coregulators (*e.g.* PGC-1α) are translocated to the nucleus or mitochondria and modulate transcription of nuclear- and mitochondrial genes important for skeletal muscle remodelling, *e.g.* mitochondrial biogenesis. *Created with BioRender.com*.

PGC-1α has been shown to coordinate the expression of many nuclear-encoded transcription factors important for energy metabolism and mitochondrial biogenesis (Baar 2004; Wu et al. 1999; Lin et al. 2005; Finck & Kelly 2006), *e.g.* mitochondrial transcription factor-A (TFAM), nuclear respiratory factor (NRF) -1, and estrogen-related receptor alpha (ERRα) and -gamma (ERRγ) (Pilegaard et al. 2003; Norrbom et al. 2004; Perry et al. 2010; Arany et al. 2008; Huss et al. 2002; Wu et al. 1999). NRFs modulate the expression of OXPHOS proteins and PPARs modulate gene expression of factors involved in lipid metabolism (Wu et al. 1999; Scarpulla 2002; Kelly & Scarpulla 2004; Gleyzer et al. 2005; Huss & Kelly 2004; Gilde & Van Bilsen 2003). TFAM, via NRF activation, is translocated to the mitochondria and regulates transcription of the mtDNA that encodes respiratory proteins as well as proteins important for mtDNA replication and transcription (Gordon et al. 2001; Hood 2001; Wu et al. 1999). Collectively, PGC-1α coordinate the expression of genes important for the early stages of mitochondrial biogenesis leading to improved skeletal muscle oxidative capacity.

Another protein shown to be involved in the regulation of skeletal muscle mitochondrial biogenesis is tumor protein p53 (p53) (Saleem et al. 2011; Li et al. 2010; Wang et al. 2010;

Granata et al. 2017). It has been shown to act as a transcription factor coregulated by PGC-1α, as well as regulating expression of PGC-1α by binding directly to the PGC-1α promoter (Aquilano et al. 2013). The p53 protein is activated (phosphorylated) upon muscle contraction and metabolic stress leading to, similar to PGC-1α, translocation to the nucleus and mitochondria and subsequent transcription of genes important for metabolism (Saleem & Hood 2013; Saleem et al. 2014; Oren 1999).

Other important factors in the complex network of molecular factors controlling the adaptation to exercise are mitochondrial transcription factor B1 and B2 (TFB1M, TFB2M), and NRF-2. Additionally, LIPIN-1 has been shown to coregulate gene transcription together with PGC-1α and PPARγ and it is shown to regulate exercise induced mitochondrial adaptation in rodents (Higashida et al. 2008; Reue & P. Zhang 2008; Kim et al. 2013). ERRα coactivate, together with PGC-1α, the induction of vascular endothelial growth factor (VEGF) transcription and PGC-1α induces the expression of ERRα itself (Arany et al. 2008; Thom et al. 2014). Also, coactivation of NRF-1 and/or ERRα increase transcription of genes important for mtDNA replication and oxidative metabolism (*e.g.* the OXPHOS protein succinate dehydrogenase (SDH)) (Kelly & Scarpulla 2004; Scarpulla 2011).

In addition to factors activating and enhancing transcription, repressors and corepressors are important regulators of transcription and are involved in the regulation of skeletal muscle adaptation to exercise. For example, nuclear receptor interacting protein-1 (RIP140) has been reported to affect NRF-1 and ERRα transcription and activity opposite to PGC-1α, *i.e.* inhibit transcription (Hallberg et al. 2008; Qi & Ding 2012). Also, the nuclear receptor corepressor 1 (NCoR1) has been proposed to supress genes that are induced by PGC-1α (Yamamoto et al. 2011; Pérez-Schindler et al. 2012) and the transcriptional corepressors retinoblastoma protein (Rb) and retinoblastoma-like 1 (p107), members of the retinoblastoma susceptibility family of proteins, are involved in the control of muscle metabolism and cellular homeostasis through repression of gene expression (Fajas 2013; Ciavarra & Zacksenhaus 2010; Blanchet et al. 2011; Petrov et al. 2016; Scimè et al. 2005; Scimè et al. 2010).

1.3.2 PGC-1α variants

During the past decades, several PGC-1α variants have been described (Miura et al. 2008; Y. Zhang et al. 2009; Ruas et al. 2012; Chinsomboon et al. 2009; Yoshioka et al. 2009), but

the nomenclature is rather confusing. Early reports showed truncated PGC-1α proteins in skeletal muscle (Kakuma et al. 2000; Baar et al. 2002), later three splice variants (PGC-1α-a, -b, and -c) were described in mice (Miura et al. 2008), where two were related to the activation of an alternative promoter (transcription from exon 1b, instead of exon 1a). Additionally, another variant was discovered that via 3´ splicing resulted in a shorted, truncated, protein called N-truncated PGC-1α (NT-PGC-1α) (Y. Zhang et al. 2009). Later, it was shown that exercise induced expression of PGC-1α from the two exons (1a and -b) in human skeletal muscle (Norrbom et al. 2011). Also, four different PGC-1α variants where characterised (PGC-1α1, a2, a3, and a4), where PGC-1α4 were described as a truncated variant transcribed via the alternative promotor from exon 1b (Ruas et al. 2012). Hence, NT-PGC1-α originates from the proximal promoter (exon 1a) and PGC-1α4 originates from the alternative promoter (exon 1b). Notably, the truncated splice variants result in shorter proteins influencing which specific transcription factors that are coactivated and the ultimate physiological effect (Y. Zhang et al. 2009). It was proposed that PGC-1α4 controls muscle hypertrophy without coactivation of PGC-1α targets involved in mitochondrial biogenesis or angiogenesis (Ruas et al. 2012).

1.3.3 Transcriptional response after exercise training

Exercise affects the whole human body in various ways, largely dependent on *i.e.* the type of exercise performed and the training status of the individual performing the exercise. There are hundreds, or even thousands, of proteins important for the regulation of skeletal muscle adaptation and adaptation maintenance.

Over an exercise training period, many of the transient messenger ribonucleic acid (mRNA) responses that follow each exercise bout gradually contribute to the accumulation of translated proteins eventually resulting in *e.g.* increased mitochondrial density (Pilegaard et al. 2003; Pilegaard et al. 2000; Perry et al. 2010; Hawley et al. 2014; Hoppeler et al. 2011; Egan & Zierath 2013; Coffey & Hawley 2007). It is widely acknowledged that the performance improvement with exercise training is non-linear with a relatively faster pace initially and a gradual plateauing off after weeks and months into the training period. It is suggested that this ‘plateau effect’ is partly due to a blunting of the molecular responses with exercise training (Fischer et al. 2004; Pilegaard et al. 2003; Tunstall et al. 2002; Lindholm, Fischer, et al. 2014). In terms of mitochondrial biogenesis, such diminished transcriptional responses occur within two weeks of training in human skeletal muscle

concurrent with the initial increases in mitochondrial proteins (Perry et al. 2010; Schmutz et al. 2006).

The concept that the blunted transcriptional response to acute exercise seen after a period of training is due to a smaller degree of perturbations to cellular homeostasis has been addressed before (Perry et al. 2010; McConell et al. 2005; Perry & Hawley 2018). However, relatively little is known about the scope of this phenomenon since much of the prior literature has mainly focused on selected transcripts and how they are affected by exercise. There is a large need for future research in this area to understand more about the complex regulation of skeletal muscle adaptation and adaptation maintenance.

2 AIMS

The overall aim of this thesis was to expand the knowledge about the molecular mechanisms involved in human skeletal muscle adaptation to exercise and exercise training, with a special emphasis on mitochondrial biogenesis.

The specific aims were to:

Paper I Investigate the transcriptional response to acute aerobic- and resistance exercise of PGC-1α variants and whether they originate from exon 1a and/or exon 1b. Also, to elucidate if aerobic- and resistance-type exercise stimulate the expression of these variants differently.

Paper II Investigate the effects during the first 24 hours of recovery after a single aerobic exercise bout on the mRNA and protein levels of PGC-1α variants, and selected factors in the surrounding molecular network.

Paper III Determine the steady-state mRNA and protein levels of Rb and p107 following three weeks of high-intensity interval training and if early changes in markers of skeletal muscle oxidative capacity were related to the expression of these transcriptional corepressors.

Paper IV Investigate the phenomenon of transcriptional-level blunting of the acute exercise response in human skeletal muscle over the course of a three-week high-intensity interval training intervention. Also, to compare the transcriptional response to acute exercise and exercise training.

3 METHODOLOGY

3.1 PARTICIPANTS, EXPERIMENTAL PROTOCOLS AND TISSUE SAMPLING

Participants were given both oral and written information about the experimental procedures and written informed consent was obtained before inclusion. All participants completed a questionnaire and non-smoking participants, free of disease not taking any medication, were included. All experimental procedures were approved by the Regional Ethical Review Board, Stockholm, Sweden (Paper I and II) or the Research Ethics Board at York University, Toronto, Ontario, Canada (Paper III and IV), and conformed to the Declaration of Helsinki.

Participants were asked to refrain from caffeine, alcohol and acetylsalicylic acid, as well as from all other forms of exercise, during the intervention. Participants were allowed to drink water *ad libitum* but sports drinks were not permitted as the carbohydrate content may affect the measurements performed on the muscle samples. For participant baseline characteristics and overview of study designs, see Table 1.

Table 1. Summary of participant baseline characteristics and study designs in papers I-IV. Data is presented as mean ± SD. EG: exercise group, CG: non-exercising control group. VO_2peak: peak oxygen uptake.

	Paper I		Paper II	Paper III	Paper IV
Exercise protocol	**Aerobic exercise** 60 min cycling	**Resistance exercise** 4 × 10 reps leg-press	**Aerobic exercise** 60 min cycling	**High-intensity interval training** 10 × 4 minutes cycling, 2 min rest between intervals, 9 exercise bouts over three weeks.	
Biopsy time points	Pre 2 hrs post	Pre 2 hrs post	Pre 30 min, 2, 6, 24 hrs post	Pre 1st, 5th and 9th bout	Pre 1st and 9th bout 3 hrs post 1st and 9th bout
Participants	*n* = 8	*n* = 8	EG: *n* = 15, CG: *n* = 5	*n* = 11	
Age (years)	27 ± 3	23 ± 3	EG: 25 ± 3 CG: 24 ± 2	25 ± 3	
Height (cm)	179 ± 12	181 ± 4	EG: 175 ± 8 CG: 177 ± 11	180 ± 6	
Weight (kg)	79 ± 12	75 ± 11	EG: 73 ± 11 CG: 69 ± 7	76 ± 11	
Body Mass Index (kg/m^2)	25 ± 2	23 ± 3	EG: 24 ± 3 CG: 22 ± 1	23 ± 3	
VO_2peak ($ml \times kg^{-1} \times min^{-1}$)	49 ± 7	51 ± 6	EG: 45 ± 6 CG: 48 ± 6	52 ± 6	

3.1.1 Aerobic exercise (Paper I and II)

Healthy men and women were randomly assigned to either an exercise group or a non-exercising control group. Prior to the intervention their peak oxygen uptake (VO_2peak) was assessed using an incremental cycle ergometer test until exhaustion. There were no significant differences between the groups regarding age, height, weight, or VO_2peak.

The participants in the exercise group performed 60 minutes of cycling exercise, at a cadence of 60 revolutions per minute (rpm), on an electrodynamically loaded cycle ergometer, while participants in the control group remained rested. During the first 20 minutes of exercise, participants cycled at a work load corresponding to 50 % of VO_2peak. After that, the work load was increased to correspond to 65 % of VO_2peak for an additional 40 minutes of exercise. Ratings of perceived exertion was measured every ten minutes using the 6-20 Borg scale (Borg 1970).

3.1.1.1 Skeletal muscle sampling

Prior to the exercise/rest, one biopsy from each leg was obtained. Succeeding biopsies (30 minutes, 2 hours, 6 hours, and 24 hours after exercise/rest) were obtained from every other leg (one biopsy per time point) and at least two centimetres apart, moving distal to proximal. Skeletal muscle samples were obtained from the middle portion of the vastus lateralis muscle using the percutaneous needle technique (Bergstrom 1975). Following local anaesthesia of the skin and fascia, a small incision was made and a skeletal muscle sample was obtained using a five-millimetre Bergström-needle with suction applied. The sample was visually inspected and blood, fat, and connective tissue were removed. The skeletal muscle sample was subsequently frozen in liquid nitrogen and stored at -80°C until analysis.

3.1.2 Resistance exercise (Paper I)

Healthy men were recruited to perform one bout of resistance exercise. Prior to the experiment, participant leg-press one-repetition maximum (1RM), at a 90-180 degrees knee angle, was determined. The load was progressively increased until the participant could not perform more than one single repetition. Participants were allowed unlimited periods of rest between sets and reached 1RM within five to six trials. Also, the participant VO_2peak was assessed during running on a treadmill with speed and incline gradually increased until exhaustion. At least five days prior to the experiment a familiarization exercise was performed with the same exercise scheduled for the experiment. At the experimental day,

participants performed a 10 minutes warm-up at a workload of 100 watts on a cycle ergometer prior to the resistance exercise bout. Thereafter, they completed 4 × 10 repetitions of leg-press at 80 % of their 1RM with five minutes of rest between sets.

3.1.2.1 Skeletal muscle sampling

Before the warm-up and exercise, a skeletal muscle biopsy was taken from the middle portion of the vastus lateralis muscle. Following local anaesthesia, a skin incision was made and four to six pieces of muscle tissue were collected, taken in different angles, using a Weil-Blakesley's conchotome (K. G. Henriksson 1979). The sample was visually inspected and blood, fat, and connective tissue were removed. The sample was subsequently frozen in liquid nitrogen and then stored at -80°C until analysis.

3.1.3 High-intensity interval training (Paper III and IV)

Eleven healthy men completed an incremental VO_2peak test on an electrodynamically loaded cycle ergometer. Participants also performed a practice exercise session at least three days after the VO_2peak test consisting of five to six four-minute cycling intervals with the same workload as the first bout of the experimental exercise.

One to two weeks after the tests participants performed nine exercise bouts, over the course of three weeks, of HIIT consisting of 10 × 4 minutes of cycling at a self-selected cadence of 80-95 rpm, with two minutes of rest between intervals. Participants were individually asked to find a comfortable cadence within that range and maintain their selection throughout the study. Each participant's heart rate reached steady-state during the final two minutes of intervals six to ten, averaging 91 % (range 89 - 94, ± 1 standard deviation (SD)) of HR_{max} (~ 83 % of VO_2peak intensity). The cycling workload was adjusted throughout the nine exercise bouts, as fitness improved as shown by decreases in steady-state heart rate, to maintain the same percentage of HR_{max}. VO_2peak was reassessed 48-72 hours after the last exercise bout.

3.1.3.1 Skeletal muscle sampling

Skeletal muscle samples were obtained from the middle portion of the vastus lateralis muscle at the first, fifth, and ninth exercise session using a spring-loaded 14-gauge disposable needle after local anaesthesia. To puncture the skin, and guide the needle, a 12-gauge cannula was used. Four to five cuts were made with the needle rotated ~30 degrees between each cut. The biopsies were visually inspected and blood, fat, and connective

tissue were removed, and the samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

3.1.4 Skeletal muscle primary myoblasts (Paper I)

For extraction of myoblasts, parts of a skeletal muscle biopsy (~40 mg) from the aerobic exercise study were put in sterile phosphate-buffered saline containing 1% penicillin-streptomycin and stored at 4°C overnight. Samples were then washed, minced, and dissociated enzymatically in trypsin EDTA at 37°C, with 5 % CO_2 and gentle agitation for 20 minutes. Undigested tissue was allowed to settle for five minutes, and the supernatant was collected in growth medium (DMEM-F-12), with 20 % FCS and 1 % penicillin-streptomycin. The cells were cultured in flasks, and growth medium was changed every third or fourth day until ~80 % confluency. Subsequently, cells were cultured in a low serum medium (DMEM-F-12 containing 2 % FCS and 1 % penicillin-streptomycin) to induce differentiation of myoblasts into myotubes. On day five of culture with differentiation medium, cells were treated with AICAR or no treatment (control), for 24 hours.

3.2 RNA ASSESSMENTS

3.2.1 RNA extraction

Total RNA from skeletal muscle biopsies, and primary myoblasts, was extracted by the acid phenol method using TRIzol™ reagent according to the manufacturer's instructions (Invitrogen). About 15-20 mg of muscle tissue were put in TRIzol™ reagent and homogenised using a polytron or bead homogeniser. Chloroform was added to allow the homogenate to separate into three layers. The RNA, in the upper clear aqueous layer, was precipitated with isopropanol and the final pellet diluted in RNase-free water. The RNA concentration was determined by measuring absorbance at 260 nm using NanoDrop 2000 (Thermo Scientific) and the RNA integrity was assessed by agarose gel electrophoresis and visualisation of bands in an ultraviolet transilluminator.

3.2.2 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Two µg of total RNA was reversed transcribed into complementary DNA (cDNA) using Superscript reverse transcriptase (Life Technologies) and random hexamer primers (Life Technologies or Roche Diagnostics). Reverse transcriptase polymerase chain reaction (RT-PCR) were done using cDNA diluted 1:100, forward- and reverse primers, dNTPs, and AmpliTaq Gold (Applied Biosystems). RT-PCR products were then run on an agarose gel

and visualised in an ultraviolet transilluminator. In Paper I, the bands for the different PGC-1α variants were excised and the RT-PCR products isolated and verified by sequencing (KIGene, Karolinska University Hospital, Stockholm, Sweden). See Figure 3 for description of PCR primers, amplicons and PGC-1α variants.

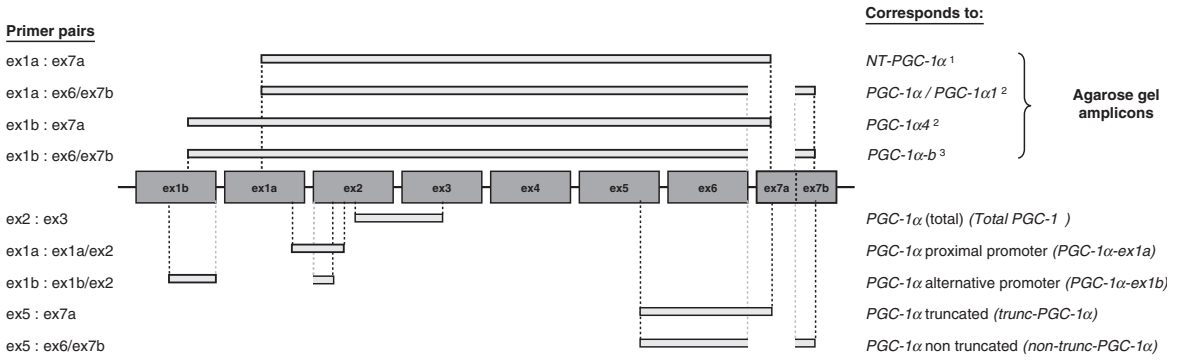

Figure 3. Schematic representation of exons 1–7 of the human PGC-1α gene. Primer pairs are depicted to the left, and the resulting splice variants measured and promoter sites are depicted to the right. Upper panel: resulting amplicons with RT-PCR. Lower panel: amplicons measured with real-time RT-PCR. Exon 1b is transcribed from the alternative promoter, and exon 1a is transcribed from the proximal promoter. Exon 7a (ex7a) is the exon insert resulting in the truncated forms of PGC-1a (trunc-PGC-1a) and exon 7b (ex7b) is present in nontruncated PGC-1a (non-trunc-PGC-1a). The corresponding names of previously described splice variants are stated to the right in the upper panel. [1]Zhang et al. (2009), [2]Ruas et al. (2012), [3]Miura et al. (2008). For primers details see Figure 1 and Table 1 in Paper II.

3.2.3 Real-time RT-PCR

Real-time RT-PCR was used for quantification of individual transcripts. This was done using TaqMan™ gene expression assay (Applied Biosystems), or SYBR™ Green fluorescent dye (Applied Biosystems) and specifically designed primers and probes, on the Applied Biosystems 7500 Fast Real-Time PCR System or CFX384 real-time PCR detection system (Bio-rad). For all quantitative PCR analyses, an endogenous control was used to control for differences in cDNA concentration. The expression of each transcript was then analysed after subtracting the endogenous control C_T value from the corresponding target C_T value. All samples from each participant were run on the same plate in one assay run when subject to comparisons. For all SYBR™ Green experiments, the specificity of the primers was verified using a melting curve and primer efficiency was tested by calculating the ΔC_T of the endogenous control and target transcripts at different concentrations. The *k*-value was less than 0.1 when ΔC_T was plotted against log(concentration) for all primer pairs used in PCR analyses.

3.2.4 RNA sequencing

RNA sequencing was used to study the global skeletal muscle transcriptome. Before the analysis, RNA quality control was done using the RNA 6000 Nano chip on the 2100 Bioanalyzer automated electrophoresis system (Agilent Technologies Inc.). The mean RNA Integrity Number (RIN) was 8.1 (range 7.5 - 8.7). The preparation of the cDNA library and the RNA sequencing was performed by the core facility for Bioinformatics and Expression Analysis at Karolinska Institutet (Stockholm, Sweden). RNA was sequenced as single-end, 50 base pairs, on the high-throughput sequencing platform Illumina HiSeq 2000, and generated an average sequencing depth across samples of ~49 million single-end reads. The expression levels of each sample were normalized as Transcripts Per Kilobase per Million (TPKM) by dividing the read count of each transcript model with its length and scaling the total per sample to one million using Kalisto V0.4.4. Quality control of raw and mapped reads was done using FastQC version 0.11.8.

3.3 PROTEIN ASSESSMENTS

3.3.1 Nuclear and Cytoplasmic Extractions

Extractions of nuclear and cytoplasmic fractions from 10-20 mg of skeletal muscle tissue were performed using NE-PER™ Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific). The muscle sample was washed in PBS and homogenised in cytoplasmic extraction reagent I buffer containing protease inhibitors (cOmplete™, Mini Protease Inhibitor Cocktail, Roche Diagnostics) using hand-rotated plastic homogenisers. The cytoplasmic fraction was extracted using cytoplasmic extraction reagent II buffer. The remaining pellet, containing the nuclear fraction, was washed in PBS before the nuclear proteins were extracted using nuclear extraction reagent buffer. Western blot, with primary antibodies specific for the nuclear protein lamin A/C and the cytoplasmic protein beta-tubulin, was used to verify the purity of the fractions.

3.3.2 Western blot

Approximately 20 mg of skeletal muscle tissue were homogenised in ice-cold cell lysis buffer. The homogenate was gently rotated at 4°C for 60 min, followed by centrifugation at 4°C for 10 min (15,000 g). Protein content was determined using a BCA assay protein assay or Bradford protein assay with BSA as standard. Equal amount of protein from all samples were subsequently boiled in Laemmli loading buffer. Denaturated protein lysates were loaded on a gradient polyacrylamide gel (Bio-rad) and separated by electrophoresis.

Proteins were then transferred onto a low-fluorescence polyvinylidene difluoride membrane (Millipore or Bio-rad). Membranes were blocked in blocking reagent (Millipore) and immunoblotted overnight at 4°C, and 45 minutes in room temperature the morning after, with primary antibodies diluted in blocking reagent specific for each protein. Subsequently, membranes were washed in PBST or TBST and incubated for one hour in room temperature with the appropriate IRDye (LI-COR Biosciences) or horseradish peroxidase conjugated (Bio-rad) secondary antibody. Membranes were visualised with chemiluminescence on photographic film or detected by infrared imaging using Odyssey SA Infrared Imaging System (LI-COR Biosciences), and quantified by densitometry using ImageJ software (National Institutes of Health) or ImageStudio (LI-COR Biosciences).

To correct for potential differences in loading amount, equal transfer and sample preparation, the target protein was related to a control protein on the same blot, except for the OXPHOS proteins when this was not possible because of size overlap. Also, the antibody supplier recommends avoidance of denaturing by heat to optimise detection of certain OXPHOS protein bands. Instead normalisation was done using controls assessed on a second blot.

3.4 STATISTICS

In Paper 1-III, the level of significance was set at 5 % ($P < 0.05$) and all statistical analyses in were performed using SPSS (IBM) or Prism (GraphPad Software). Data is presented as mean ± SD or standard error of the mean (SEM).

Paper I - The delta cycle threshold (ΔC_T) value, obtained by subtracting the reference mRNA C_T value from the target mRNA C_T value, were used for statistical analysis of mRNA content. For expression comparisons, *PGC-1α-ex1a* was subtracted from *PGC-1α-ex1b* and *non-trunc-PGC-1α* was subtracted from *trunc-PGC-1α.*. The quantity of each target was then determined using the $2^{-\Delta CT}$ method which provides the amount of target mRNA as a ratio to the reference mRNA for each sample. A mixed model two-way analysis of variance (ANOVA) was used to evaluate the effect of one bout of endurance or resistance exercise, and AICAR-stimulation or control in cell culture experiments, on mRNA content. Planned comparisons (Student's *t*-tests) were made to locate differences corresponding to interactions or when no interaction was found, to identify differences corresponding to significant main effects in the ANOVA models.

Paper II - Total protein and mRNA content in response to one bout of endurance exercise, or non-exercising control, were analysed using linear mixed models (LMM). The ΔC_T values were used for statistical analysis of mRNA content. Pairwise comparisons within groups using Fisher's least significant difference (LSD) test were used as *post hoc* analysis. Protein content in nuclear and cytoplasmic fractions, in response to one bout of endurance exercise, were analysed using one-way repeated-measures ANOVA. For protein content analysis, reference protein was subtracted from the target protein. Outliers, defined as observations < Q1 - (1.5 × IQR) or > Q3 + (1.5 × IQR), were excluded from the analysis (Q: quartile, IQR: interquartile range).

Paper III - One-way ANOVA was used to compare the steady-state pre-exercise protein content and mRNA at the first, fifth, and ninth HIIT session, followed by Tukey's *post hoc* test. The ΔC_T values were used for statistical analysis of mRNA content. Correlations were estimated using the Pearson's correlation coefficient, *i.e.* assuming linear relationship between pairwise variables.

Paper IV – Differential gene expression analysis was performed on the mapped read counts using the edgeR package in R, correcting for common, trended and tagwise disparity with the formula log TPKM = 0 + Time × Bout with each participant as block-factor to account for the repeated measures design. The analysis rendered p-values, two-base logarithmic fold change (log_2FC) differences and false discovery rate (FDR) values for the contrasts acute exercise 1st bout, acute exercise 9th bout, exercise training (steady-state), and acute exercise 9th bout *vs.* acute exercise 1st bout. For all contrasts an FDR of < 1 % was considered significant. Pathway analysis was performed using gene-set enrichment analysis (GSEA) where pathway annotation of genes was retrieved from WikiPathways current as of December 2018 (Slenter et al. 2018). For both overrepresentation and gene-set enrichment an FDR of < 5 % was considered significant. Pathway-level comparison of the different conditions was calculated using two-sided Wilcoxon Signed-Rank tests on empirical cumulative distributions where an FDR of < 5 % was considered a significant difference.

4 RESULTS AND DISCUSSION

Three different human exercise experimental models were employed to study the transcriptional and protein expressional response of factors involved in skeletal muscle adaptation to exercise and exercise training.

4.1 WHOLE-BODY RESPONSE TO THREE WEEKS OF HIIT

With three weeks of HIIT, both mean (±SD) power output during the training sessions and mean (±SD) VO_2peak increased significantly with 9 % (baseline; 240 (43) watts and 52 (6) ml × kg^{-1} × min^{-1}, respectively, Figure 4) following nine sessions, over three weeks of HIIT (Paper III and IV). This is somewhat less than what Perry *et al.* (2010) showed (VO_2peak increase 12 %, power output increase 18 %) when participants performed essentially the same exercise protocol except that they completed seven sessions instead of nine (Perry et al. 2010). This difference can partly be explained by that the participants in the study by Perry et al. had a lower VO_2peak before the intervention. However, in general, the VO_2peak improvement seen in the present HIIT study is what can be expected over the selected time period in non-elite trained individuals (Milanović et al. 2015).

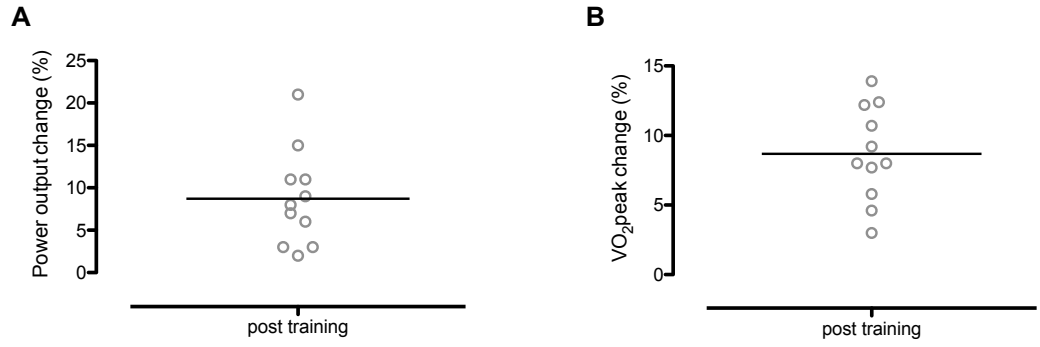

Figure 4. Physiological improvements after three weeks of HIIT. Individual (circles, *n* = 11) and group mean (line) for (A) power output change (%) comparing the first and ninth bout of exercise and (B) VO_2peak change (%) after exercise training.

4.2 TRANSCRIPTOME RESPONSE AFTER ONE BOUT OF HIIT

An acute bout of HIIT resulted in an extensive effect on the muscle transcriptome three hours after exercise with 2,320 differentially expressed genes (FDR < 1 %, Paper IV), corresponding to almost 20 % of all genes presumed to be expressed in human skeletal muscle (Lindholm, Huss, et al. 2014). Out of the 2,320, 62 % were upregulated and out of those, 10 % were strongly induced (mean $log_2FC \geq 2$). Of the differentially expressed

genes, 38 % were downregulated with almost 10 % being decreased with $\log_2FC \leq -1$. None of the downregulated genes were reduced more than $\log_2FC$ -1.9. Hence, the overall pattern of differentially expressed genes after one bout of HIIT were, as expected, that the majority were upregulated by acute exercise (Pacheco et al. 2018).

Among the top 25 upregulated genes were those coding for transcriptional regulators related to metabolism (*e.g.* PGC-1α, PDK4, and SLC16A6), apoptosis and cell differentiation/proliferation (FOS), oxidative stress (MAFF and JUNB), ECM modulation and angiogenesis (*e.g.* THBS1, ADAMTS4, TNC, and MYC), as well as other nuclear receptors and transcription factors (*e.g.* EGR1, EGR2, EGR3, and NR4A3). The majority of transcripts included in metabolism related pathways (*Energy-, Carbohydrate, Lipid,* and *Amino acid metabolism*) were upregulated. The only pathway with the majority of genes being downregulated was *mTOR signaling pathway*.

4.3 TRANSCRIPTOME RESPONSE TO ACUTE EXERCISE AFTER EXERCISE TRAINING

After three weeks of HIIT, 947 genes were differentially expressed after acute exercise. When comparing differentially expressed genes after the first and ninth bout, 882 differentially expressed genes were overlapping (Figure 5, red sector). More than 60 % of the genes differentially expressed after the first bout were exclusive for the first bout. There were 1,435 genes regulated by the first exercise bout only (Figure 5, green sector) and 65 genes regulated by the ninth exercise bout only (Figure 5, purple sector). The number of significantly upregulated genes at the first and ninth exercise bout were 1,429 and 667, respectively, and the number of downregulated genes were 891 and 275, respectively.

The transcriptional response to acute exercise was strikingly reproducible before and after three weeks of training. In fact, correlating the $\log_2FC$ between the first and ninth exercise bout gave a Pearson correlation coefficient of 0.9 illustrating that both directionality and magnitude were very similar. This indicates that the overall transcriptional response to acute exercise is reproducible and that essentially the same genes and pathways are activated after each exercise bout, independent of training status. However, the expression was attenuated after the last exercise bout, where the fraction of differentially expressed transcripts were 40 % of that found after the first exercise bout (947 compared to 2,320). Also, the beta-coefficient (*i.e.* slope) were 0.63, showing that the overall response is larger after the first exercise bout than after the ninth.

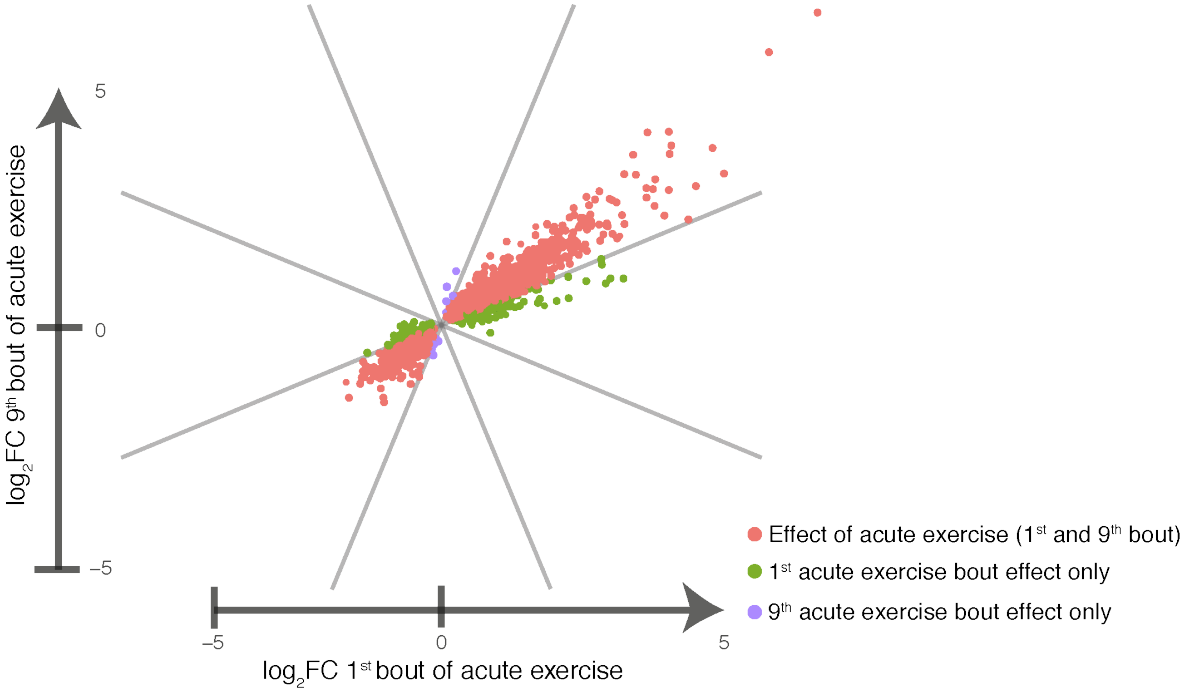

Figure 5. Transcriptional response to acute exercise at the first and ninth bout after three weeks of HIIT in human skeletal muscle. BiComp correlation plot with the continuous relationship between genes differentially expressed (FDR < 1 %) at the first *or* ninth acute bout of exercise. For each individual gene, log_2FC for the first bout (x-axis) were plotted against log_2FC for the ninth bout (y-axis), thus creating a polar coordinate system illustrating similarities/dissimilarities of transcriptional response between the two conditions. The resulting circle is divided into 8 sectors, each covering 45 degrees of the circle. Red sector: genes with similar effect in the two conditions; Green sector: genes regulated by the first, but not by the ninth bout, of exercise; Purple sector: genes regulated by the ninth, but not by the first bout, of exercise.

Correlating the fold changes of differential expressed genes at the first and ninth exercise bout revealed that the blunting of the transcriptional response to acute exercise after training varied largely between transcripts (Figure 5). A large portion of the regulated genes had a similar magnitude or displayed only minor blunting effects of change at the ninth bout compared with the first bout. However, a significant proportion of the transcripts were diminished enough after the ninth bout to end up in the green sector (Figure 5), meaning that they were affected by the first exercise bout only.

Surprisingly, there were a few genes that were differentially expressed after the last exercise bout only. A possible explanation could be that these genes, responding in 'trained' muscle only, are coupled to a later phase of adaptation and to maintenance of the muscle tissue adaptation. However, these genes constitute only about 1 % of the total number of significantly regulated genes. Since the FDR was set to 1 % it could be speculated that some of these differentially expressed genes are actually false discoveries.

When comparing well-trained individuals with those that are sedentary, this transcriptional blunting phenomenon has shown to be present even when the less-trained individuals complete only 50 % of the exercise protocol performed by well-trained athletes (Yu et al. 2003). Also, the mRNA response after an acute bout of intense exercise is shown to be less pronounced after six weeks of endurance training (Schmutz et al. 2006).

One possible reason for the attenuated mRNA response after acute exercise with exercise training is a lower local skeletal muscle tissue stimulus at the end of the exercise intervention period. Also, in the present study the workload was adjusted to correspond to 91 % of HR_{max} across training sessions, meaning that the exercise intensity was determined more by central factors than by peripheral stress on the muscle. Stimuli, such as metabolic perturbation and oxidative stress, are presumed to be relatively lower at the last exercise bout compared to the first bout since the muscle has adapted relatively more than the cardiorespiratory system (Egan & Zierath 2013). However, when comparing this to resistance exercise where the workload can be adjusted more easily to stress the muscle with the same relative load, the transcriptional response after acute exercise was still attenuated after training (Damas et al. 2018; Nader et al. 2014).

4.3.1 Pathways regulated after acute exercise

The comparison of the transcriptomic response at the first and ninth exercise bout was further analysed on the pathway level (Paper IV). A large number of pathways were found to be significantly different between bouts. Out of the 111 significantly regulated pathways by the first exercise bout, 66 were also differentially regulated by the ninth bout. Rather surprisingly, the *Electron Transport Chain (OXPHOS system in mitochondria)* pathway was unchanged at the first bout and downregulated at the last. The *TCA Cycle (aka Krebs or citric acid cycle)* pathway was downregulated by both the first and ninth exercise bout. Out of the pathways related to energy metabolism, *Glycolysis and Gluconeogenesis* was the only significantly upregulated pathway by the first bout. This is in line with another study that did not find groups of GO terms related to mitochondrial biogenesis, oxidative reactions, or carbohydrate metabolism after acute exercise (Popov 2018). An additional study reported induction of the OXPHOS pathway at steady-state after six weeks of exercise training (Timmons et al. 2010). However, when analysing individual genes, they found that only a few genes where differentially expressed. They concluded that OXPHOS genes are not extensively regulated after exercise training in human skeletal muscle. The *Energy Metabolism* and *Oxidative Stress* pathways were clearly upregulated by the first and

ninth bout meaning that these pathways respond to acute exercise regardless of training status. However, the fold change increases were lower at the ninth bout. It could be speculated that an extended intervention period would blunt the response even further.

The *PPAR Alpha Pathway* was significantly regulated at steady-state after three weeks of training but not after the first bout of exercise (Figure 7B). This is in line with the suggestion by Popov et al. (2018), that 'trained' skeletal muscle gene expression after acute exercise mainly consists of regulatory genes. They showed that one of the most significantly regulated GO terms in 'trained' muscle after acute exercise is related to regulation of transcription and proposed that the protein activity of transcriptional regulators are important in the initial phase of recovery, rather than the mRNA expression increasing the protein levels (Popov 2018).

4.4 TRANSCRIPTOME RESPONSE AFTER ACUTE EXERCISE AND EXERCISE TRAINING

With three weeks of exercise training (steady-state), 119 genes were differentially expressed, with 67 % being upregulated and 33 % downregulated (Paper IV). A minority of these genes was similarly regulated by acute exercise (Figure 5, red sector).

The transcriptional response to acute exercise was contrasted with the steady-state response to exercise training. There were 119 differentially expressed genes at steady-state after three weeks of HIIT, which corresponds to only 5 % of what was differentially expressed after the first bout. Out of the 2,320 genes significantly regulated after the first exercise bout only 16 genes were differentially expressed at steady-state after training. Hence, less than 1 % of the total number of differentially regulated genes after an acute bout of HIIT were regulated at steady-state after training.

When comparing the acute transcriptional response with the steady-state response to exercise training (Figure 6) it seems plausible that the adaptation to training is largely orchestrated by other genes than those responding to an acute bout of exercise. The possible biological significance of this is that some genes are involved in the skeletal muscle remodelling and other genes are vital for the maintenance of the skeletal muscle tissue adaptation. This was further supported by the pathway analysis, see discussion below.

It is somewhat paradoxical that a few genes respond in opposite directions when comparing the acute response at the first exercise bout with the steady-state effect (Figure 6, blue). However, these genes are rather close to the centre of the plot implying they are not

particularly regulated. Also, genes that are vastly regulated following the first exercise bout are not regulated in the opposite direction with training. Rather, they are 'rotated counter clockwise' towards being regulated by acute exercise only (Figure 6, green).

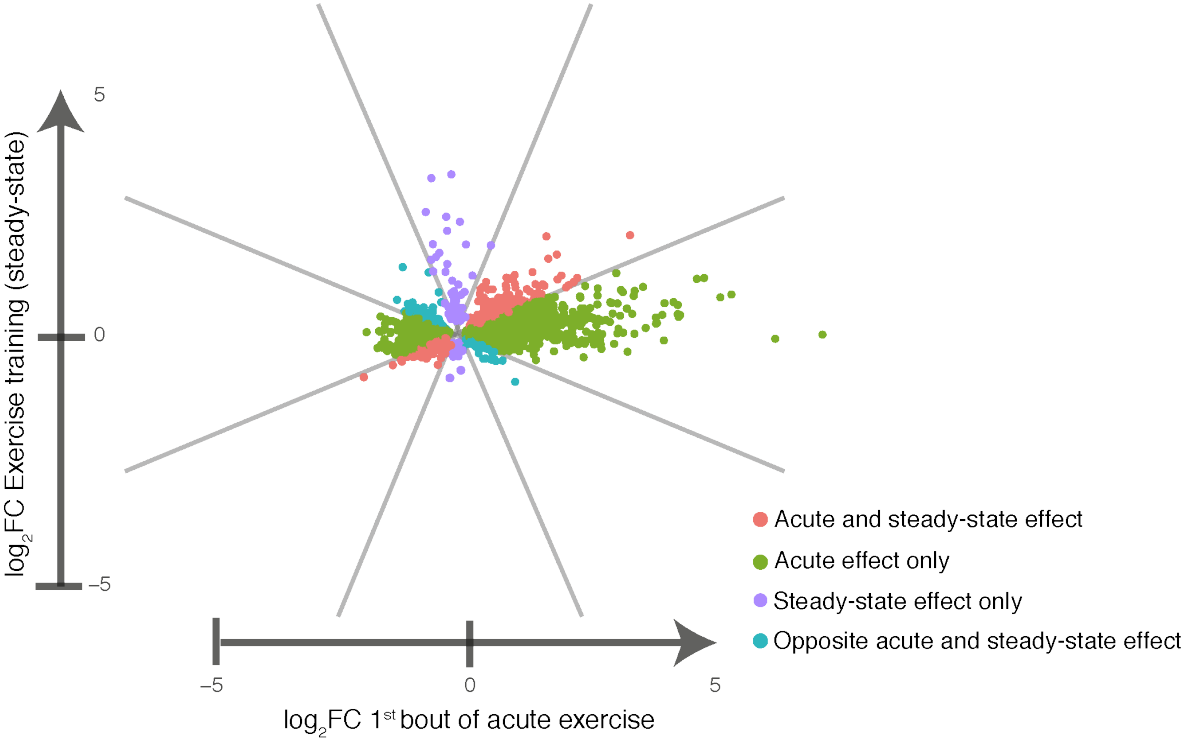

Figure 6. Transcriptional response to acute exercise and three weeks of HIIT in human skeletal muscle. BiComp correlation plot with the continuous relationship between genes differentially expressed (FDR < 1 %) at the first acute bout of exercise (x-axis) *or* at steady-state after exercise training (y-axis). For each individual gene, log_2FC for the first bout (x-axis) were plotted against log_2FC for steady-state after exercise training (y-axis), thus creating a polar coordinate system illustrating similarities/dissimilarities of transcriptional response between the two conditions. The resulting circle is divided into 8 sectors, each covering 45 degrees of the circle. Red sector: genes with similar effect in the two conditions; Green sector: genes regulated by the first exercise bout but not by exercise training; Purple sector: genes regulated by exercise training but not by the first exercise bout; Blue sector: genes regulated in opposite directions in the two conditions.

4.4.1 Pathways regulated after acute exercise and exercise training

Almost all (95 %) of the differentially regulated pathways after the first bout of exercise were significantly different at steady-state after training (Paper IV). Hence, a larger proportion of pathways regulated by acute exercise, are not regulated with training than for the reverse. This strengthens the hypothesis that the steady-state transcriptome includes factors involved the maintenance of skeletal muscle adaptation, rather than tissue remodelling.

At steady-state after exercise training, some of the most induced pathways (based on log_2FC) different from the first bout were *e.g. Nuclear Receptor Meta-Pathway, Adipogenesis, TGF-beta Signalling Pathway, Focal Adhesion-PI3K-Akt-mTOR-signaling pathway*, and *Human Complement System.* The third most upregulated pathway by acute exercise is *Hypertrophy Model.* This pathway was significantly downregulated by exercise training. Of pathways significantly regulated after acute exercise *Oxidative* Stress and *Glycolysis and Gluconeogenesis* are two of the most induced pathways after exercise training. An overall pattern of the gene expression at steady-state after training is that the response is smaller and less scattered compared to after acute exercise (Figure 7).

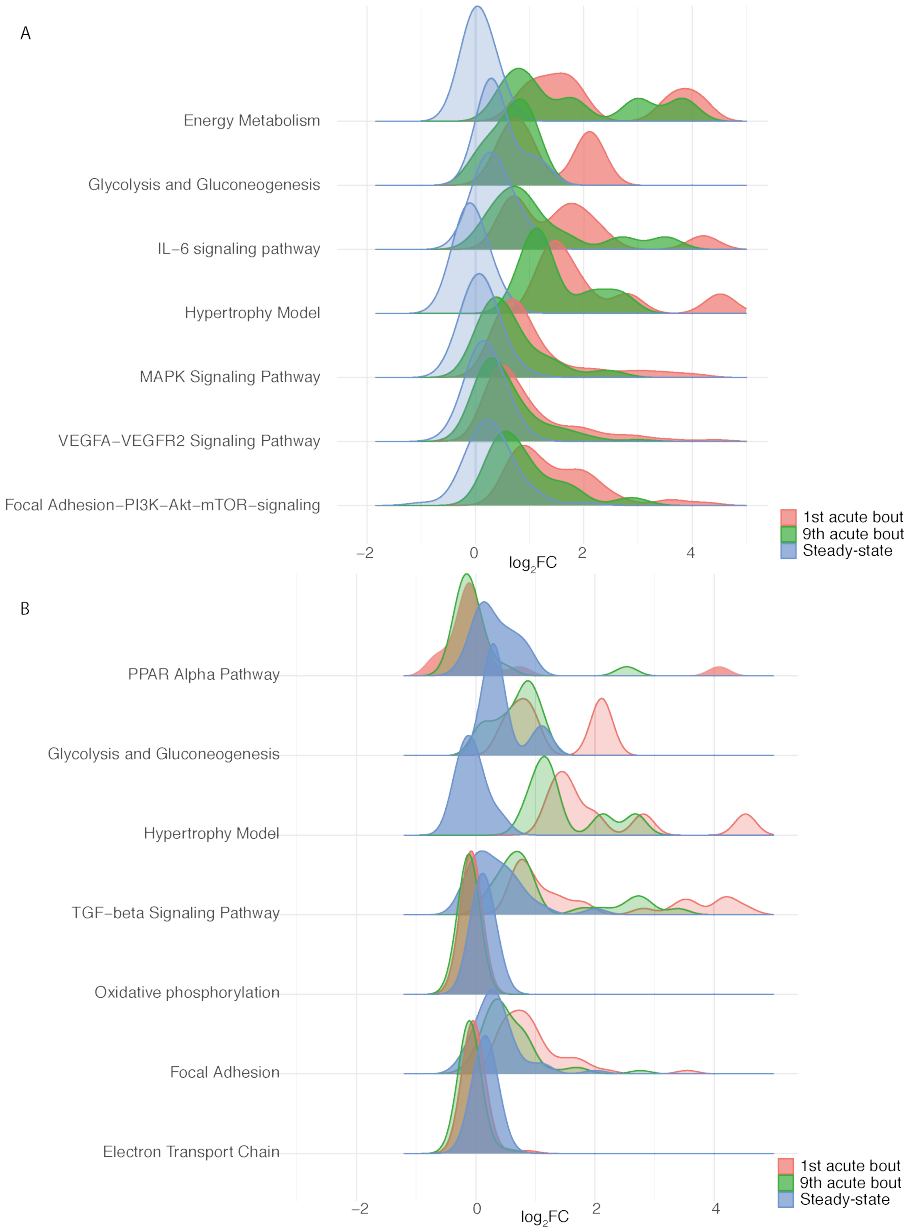

Figure 7. Pathways regulated by acute exercise and/or exercise training. Ridgeplots illustrating log_2FC densities of all genes belonging to the respective pathway and separated based on condition (Red: first acute bout, Green: ninth acute bout, Blue: steady-state after exercise training).

4.5 TRANSCRIPTIONAL ACTIVATORS AFTER ACUTE AEROBIC EXERCISE

Several signalling pathways have been suggested to regulate aerobic adaptations (Winder & Hardie 1996; Wu et al. 1999; Wright et al. 2007; Chinsomboon et al. 2009; Bishop et al. 2019). A selection of factors in these pathways were measured to investigate the temporal expression pattern during the first 24 hours of recovery from acute exercise (Paper II). A non-exercising control group was included to elucidate the true exercise effect on various transcriptional regulators important for mitochondrial biogenesis, as well as down-stream targets of the 'master regulator' of mitochondrial biogenesis, PGC-1α.

There are several coactivators that interact with PGC-1α, *e.g.* NRF-1, NRF-2, PPARγ, and ERRs. In line with previous studies (Pilegaard et al. 2003), neither ERRα nor NRF-1 were affected by one hour of aerobic exercise (Figure 8A and D, respectively). ERRγ mRNA was clearly affected by acute aerobic exercise and increased six hours into the recovery (Figure 8B). ERRγ is suggested to interact with AMPK as well as to be activated by PGC-1α leading to activation of genes important for mitochondrial biogenesis and β-oxidation in the mitochondria (Rangwala et al. 2010; Giguère 2008). Its involvement in aerobic adaptations is strengthen by it being more abundant in oxidative fibres than in glycolytic fibres (Narkar et al. 2011).

There was a significant difference in p53 expression between the exercise- and control group (Figure 8C). However, p53 transcription was also induced in the non-exercising control group. In the exercise group, the peak of the p53 mRNA increase was six hours into recovery, which corresponded to the only post-exercise time point were the control group did not have an altered level of p53 mRNA.

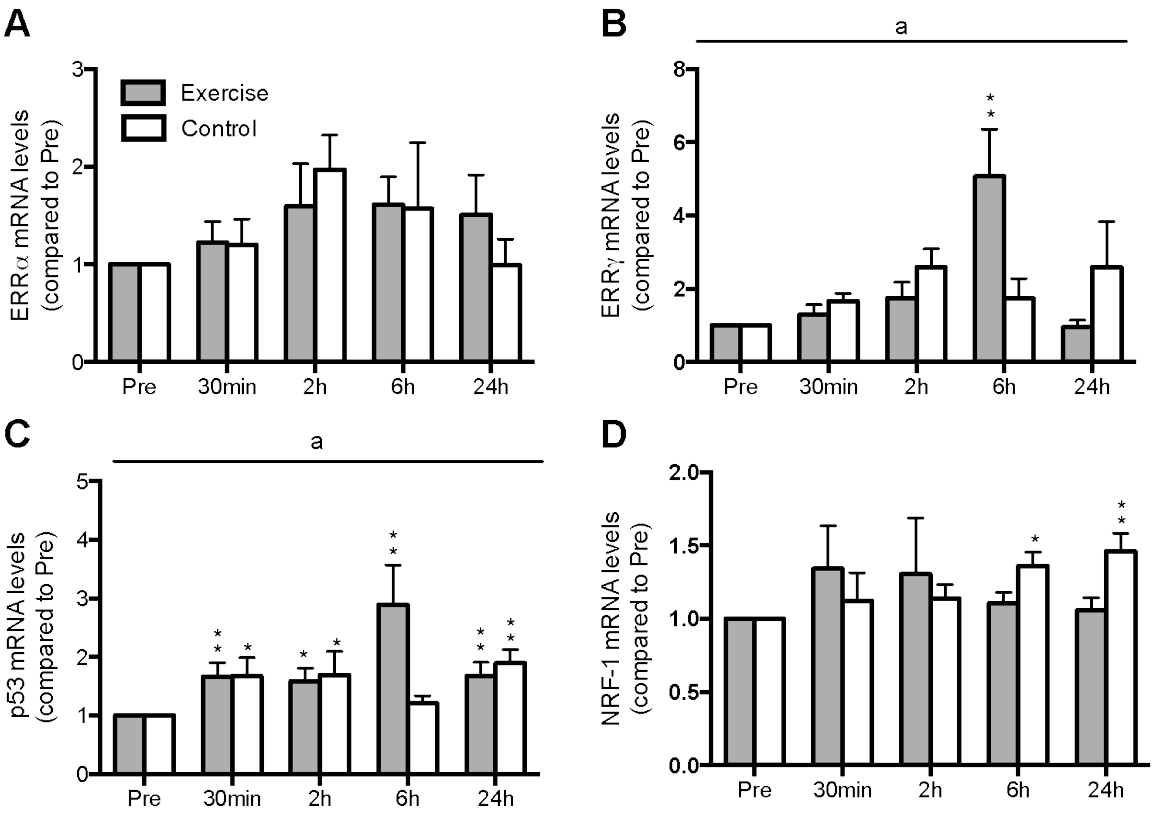

Figure 8. Skeletal muscle mRNA levels of transcriptional activators important for mitochondrial biogenesis. mRNA levels of (A) ERRα (B) ERRγ (C) p53, and (D) NRF-1, before (pre) and after (30 min, 2 hours, 6 hours, and 24 hours) one bout of aerobic exercise (n = 15 (ERRγ, n = 14)) or non-exercising control (n = 5 (ERRγ, n = 4)). $^{a}P \leq 0.05$ with factors time × group, $^{*}P < 0.05$ compared to Pre, $^{**}P \leq 0.01$ compared to Pre. Values are presented as mean ± SEM.

The expression of the lipid metabolism enzyme LIPIN-1, suggested to also act as a transcription factor with PGC-1α binding capacity (Reue & P. Zhang 2008; Kim et al. 2013), was significantly upregulated two hours into the recovery after acute aerobic exercise, and stayed elevated up to 24 hours (Figure 9A). Two alternative transcripts (LIPIN-1α and LIPIN-1β), earlier not reported to exist in human skeletal muscle, were detected (see Paper II; Figure 1) and quantified. LIPIN-1α were significantly upregulated after exercise (30 min, 6 hours and 24 hours) but LIPIN-1β were not significantly different from the control group (Figure 9B and C, respectively). The total LIPIN-1 mRNA expression largely corresponded to the expression pattern seen for the two alternative transcripts. Noteworthy, the expression of total LIPIN-1 and for the two alternative transcripts was increased in the control group at 24 hours. This could be explained by that the biopsy procedure affected the expression or the low power in the analysis with only five participants in the control group. Unfortunately, LIPIN-1 protein could not be detected in

the protein homogenates from the skeletal muscle biopsies. The role of LIPIN-1 in mediating aerobic adaptations to exercise still needs to be determined.

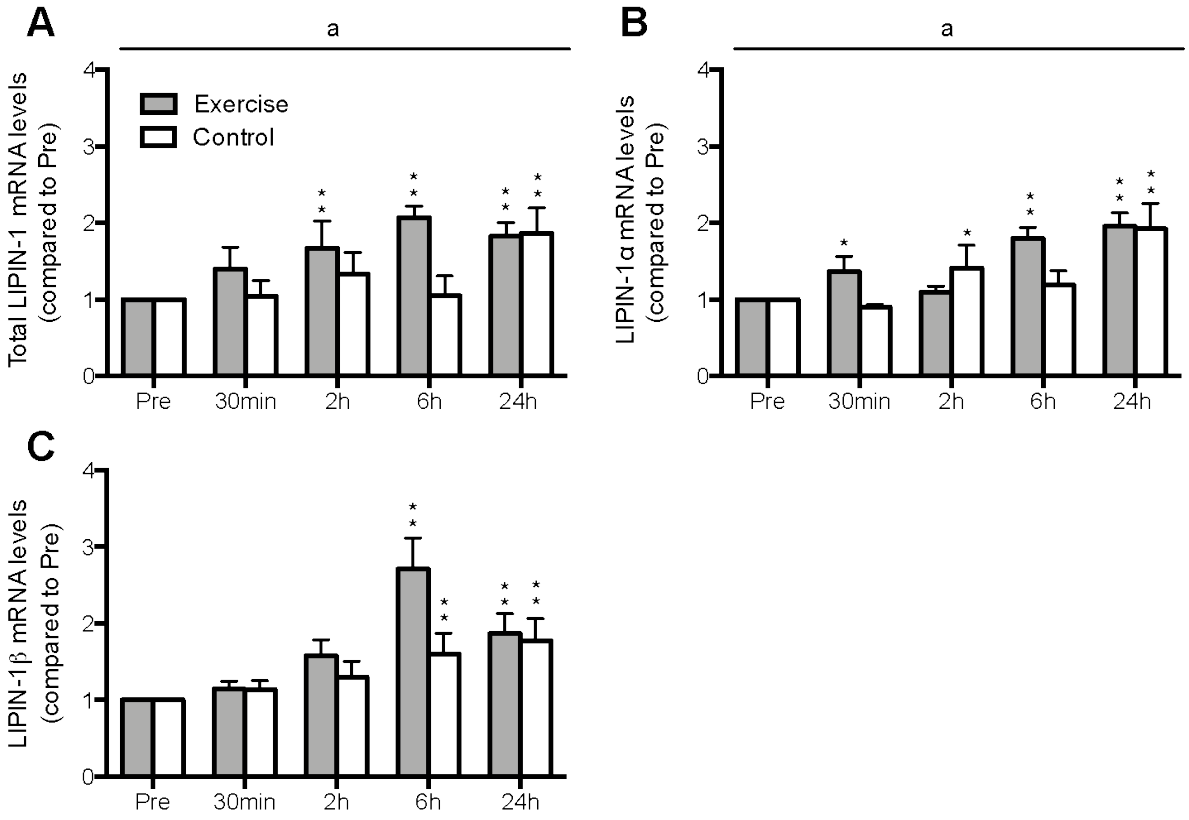

Figure 9. Skeletal muscle mRNA levels of LIPIN-1 after one bout of aerobic exercise. mRNA levels of (A) Total LIPIN-1 (B) LIPIN-1α, and (C) LIPIN-1β, before (pre) and after (30 min, 2 hours, 6 hours, and 24 hours) one bout of aerobic exercise (n = 15) or non-exercising control (n = 5). $^{a}P \leq 0.05$ with factors time × group, $*P < 0.05$ compared to Pre, $**P \leq 0.01$ compared to Pre. Values are presented as mean ± SEM.

4.5.1 PGC-1α expression after exercise and exercise training

The pathway *Energy Metabolism* was unaffected by three weeks of HIIT (Paper IV, Figure 7A in thesis). PGC-1α is one of the genes included in that pathway. Not surprisingly, PGC-1α mRNA steady-state level was not regulated by exercise training (Paper IV, data not shown). Further, twenty-four hours after an acute bout of aerobic exercise (Paper II) PGC-1α expression was almost back to baseline levels (Figure 13A). This confirms previous studies showing that this transcript is almost, or completely, back to baseline level 24 hours after acute exercise (Pilegaard et al. 2003; Perry et al. 2010; Egan & Zierath 2013; Norrbom et al. 2011).

During the first hours of recovery, PGC-1α was increased after both moderate- (Figure 12A and 13A, Paper I and II, respectively) and high-intensity exercise (data not shown, Paper

IV). However, after three weeks of training the increase was blunted (Paper IV), which is in line with previous studies showing an attenuated increase after acute exercise in PGC-1α mRNA with exercise training, both with the same relative exercise intensity (Perry et al. 2010; Fernandez-Gonzalo et al. 2013), as well the same absolute exercise intensity (Stepto et al. 2012; Morrison et al. 2015). This was phenomenon was further confirmed in a recent study with high-volume training that resulted in a complete loss of the exercise-induced increase in PGC-1α mRNA (Granata et al. 2019).

Additionally, protein levels and cellular location of PGC-1α were examined (Paper II). After acute aerobic exercise, Total PGC-1α and PGC-1α-ex1b protein levels were not significantly different compared to non-exercising controls (Figure 10A and B, respectively). However, when analysing the exercise group only there was a slight increase 24 hours post exercise of Total PGC-1α (1.2-fold) but a larger increase of PGC-1α-ex1b (3.1-fold) 30 minutes after exercise. Previous studies have shown exercise-induced PGC-1α translocation to the nucleus where it coactivates gene transcription of various factors important for the adaptation to exercise (Little et al. 2011; Safdar et al. 2011; Wright et al. 2007). To assess the timing of the PGC-1α translocation after exercise both PGC-1α and PGC-1α-ex1b were measures in nuclear and cytoplasmic homogenate fractions. However, neither Total PGC-1α nor PGC-1α-ex1b were translocated (Figure 10C and D, respectively) after one hour of moderate-intensity exercise. A succeeding study showed that ‘all-out’ sprint exercise, but not moderate-intensity exercise, translocated PGC-1α to the nucleus (Granata et al. 2017). Hence, greater exercise-intensities is probably favourable for the early molecular events preceding skeletal muscle adaptation.

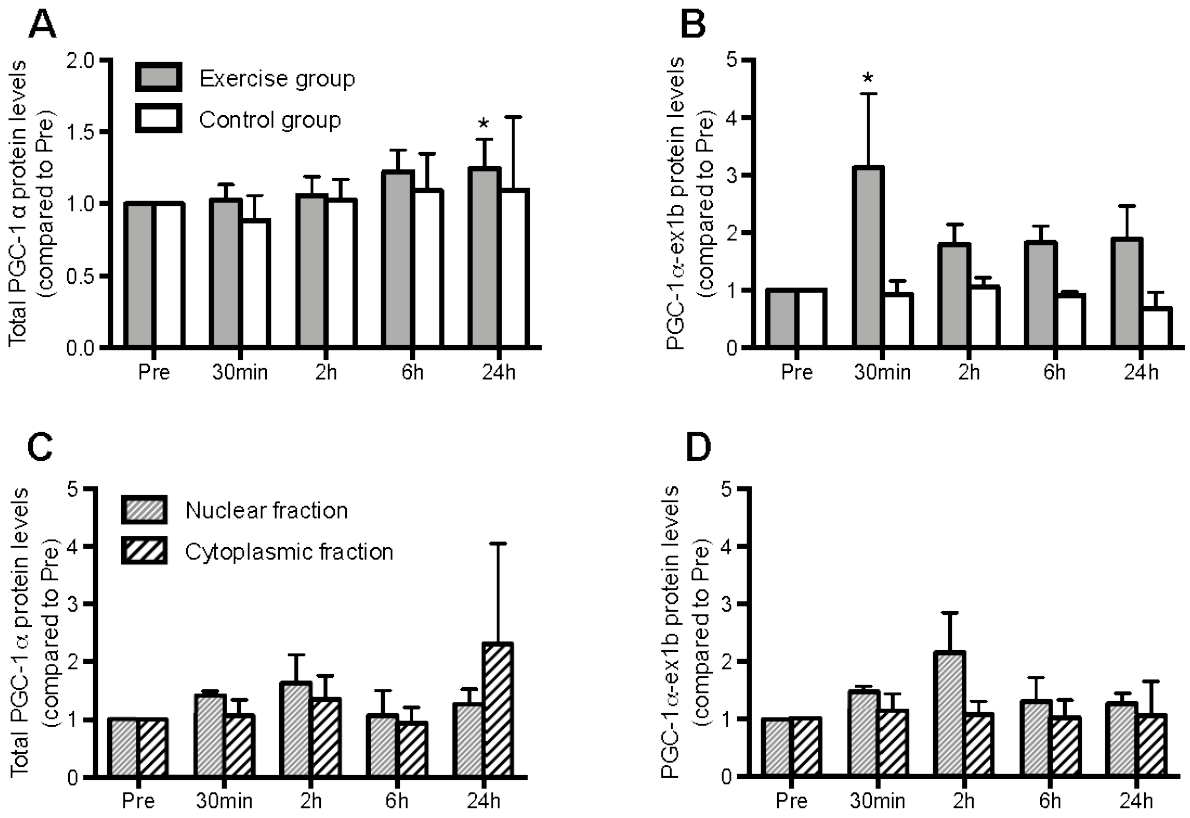

Figure 10. Skeletal muscle protein levels of PGC-1α and PGC-1α-ex1b after one bout of aerobic exercise. Total protein levels of (A) PGC-1α and (B) PGC-1α-ex1b, one bout of aerobic exercise (n = 12) or non-exercising control (n = 4). Nuclear and cytoplasmic protein levels of (C) PGC-1α and (D) PGC-1α-ex1b, before (pre) and after (30 min, 2 hours, 6 hours, and 24 hours) one bout of aerobic exercise (n = 5). $^{a}P \leq 0.05$ with factors time × group, $^{*}P < 0.05$ compared to Pre, $^{**}P \leq 0.01$ compared to Pre. Values are presented as mean ± SEM.

4.5.2 PGC-1α variants

In Paper I it was shown that at least four different PGC-1α variants were transcribed in human skeletal muscle. Both the truncated and nontruncated variants were transcribed from exon 1a and the alternative upstream located exon 1b (Figure 11, see Figure 3 for description of PCR primers, amplicons and PGC-1α variants). Important to note is that the PCR primers used in Paper I and II are not able to differentiate if the non-truncated and truncated PGC-1α variants are transcribed from exon 1a or -b since they are positioned downstream in exon 7. Due to methodological limitations, it was not possible to use a primer in exon 1 combined with a primer in exon 7 and thereby differentiate between the different variants. Hence, it was not possible to measure PGC-1α4 specifically.

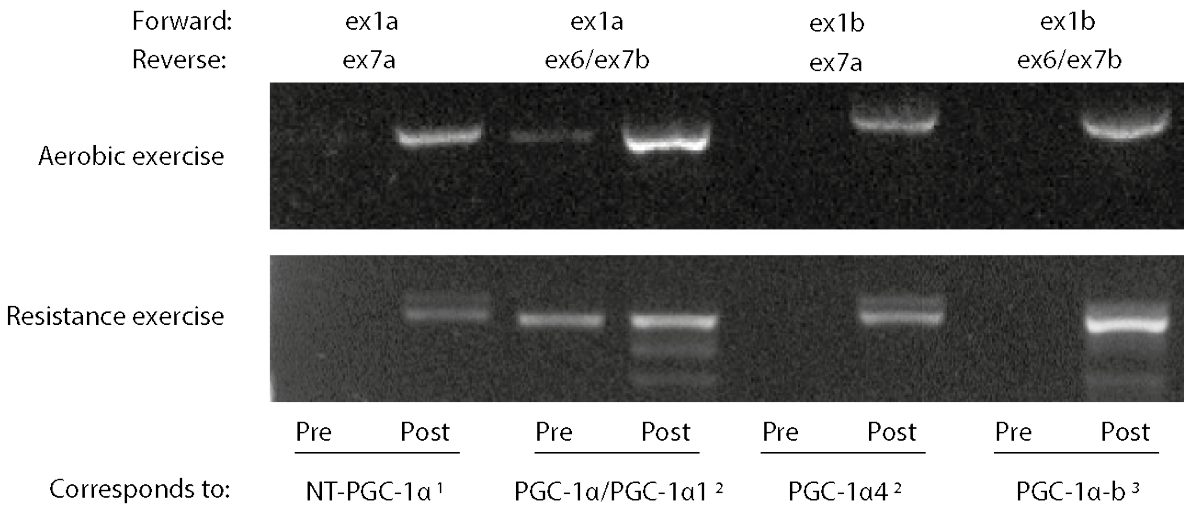

Figure 11. Skeletal muscle PGC-1α variants in human skeletal muscle. Agarose gel with RT-PCR products, from using primers designed to measure the various PGC-1α variants, of truncated and nontruncated variants of PGC-1α transcribed from two different exons. Forward primers located in exon 1a (ex1a) and exon 1b (ex1b) were combined with reverse primers for truncated (ex7a) and nontruncated (ex6/ex7b) PGC-1α variants. The bands correspond to the calculated amplicon sizes, showing that both truncated and nontruncated forms are expressed from both the proximal and the alternative promoter. The corresponding names of previously described variants are stated below the gel. [1]Zhang et al. (2009), [2]Ruas et al. (2012), [3]Miura et al. (2008).

PGC-1α4 has been reported to be highly expressed after exercise training (Ruas et al. 2012; Nader et al. 2014), but not to regulate *e.g.* mitochondrial OXPHOS genes and other known PGC-1α targets (Ruas et al. 2012). Rather, it is suggested to induce insulin-like growth factor 1 and repress myostatin, and to regulate skeletal muscle hypertrophy. However, one bout of both aerobic and resistance exercise (Paper I), induced transcription of four PGC-1α variants (trunc-PGC-1α, non-trunc-PGC-1α, PGC-1α-ex1a, and PGC-1α-ex1b) and Total PGC-1α and could thereby not confirm that there is an exercise mode specific transcript (Figure 12). There was a significant difference between the effects of the two exercise types for Total PGC-1α and trunc-PGC-1α, where the expression increases where larger after aerobic exercise than after resistance exercise. Another study comparing the effects of aerobic- and resistance exercise showed that both exercise modalities induced PGC-1α-ex1b as well at the truncated transcripts whereas only endurance exercise induced PGC-1α-ex1a expression (Silvennoinen et al. 2015). An additional study showed that resistance exercise did not specifically induce the truncated PGC-1α variants differently from aerobic- and resistance exercise combined. They showed that trunc-PGC-1α expression was even greater after concurrent exercise than for resistance exercise alone and

concluded that PGC-1α4 is not associated with exercise-induced human muscle hypertrophy (Lundberg et al. 2014).

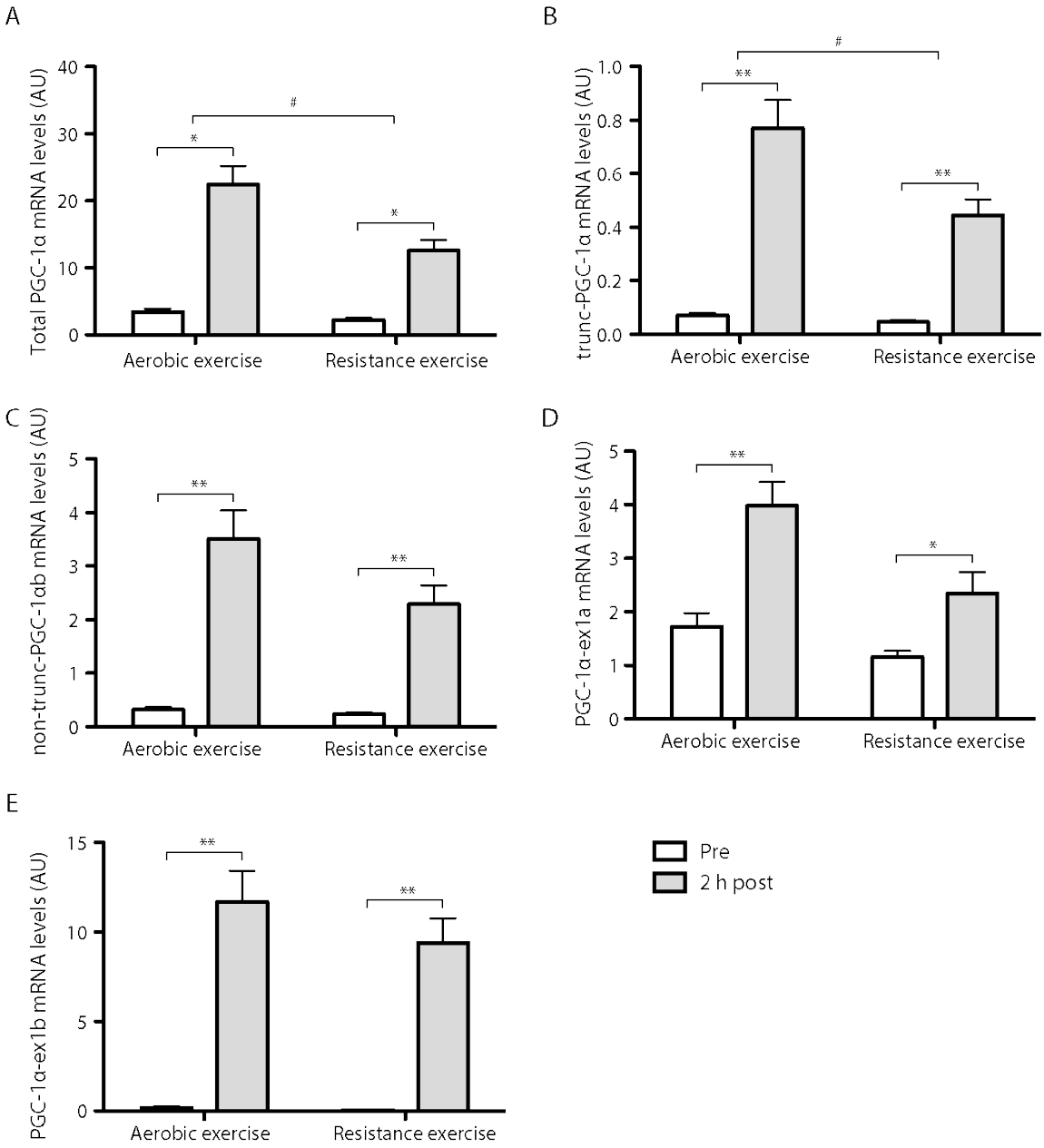

Figure 12. Skeletal muscle mRNA levels of PGC-1α variants after one bout of aerobic- or resistance exercise. mRNA levels of (A) Total PGC-1α, (B) trunc-PGC-1α, (C) non-trunc-PGC-1α, (D) PGC-1a-ex1α, and (E) PGC-1α-ex1b, before (pre) and 2 hours after (2 h post) one bout of aerobic (n = 8) or resistance (n = 8) exercise. $^{\#}P < 0.05$ aerobic- *vs.* resistance exercise, $^{*}P < 0.05$ pre *vs.* 2 h post, $^{**}P < 0.01$ pre *vs.* 2 h post. Data is presented as mean ± SEM.

When the expression of PGC-1α-ex1a in response to aerobic exercise was compared to a non-exercising control group (Paper II) instead of a resistance exercise group (Paper I), no significant difference was observed for PGC-1α-ex1a between the groups (Figure 13C).

However, the remaining variants, and Total PGC-1α, were significantly elevated 30 minutes, 2 hours, and 6 hours after aerobic exercise. Total PGC-1α and PGC-1α-ex1b were still significantly elevated after 24 hours (Figure 13).

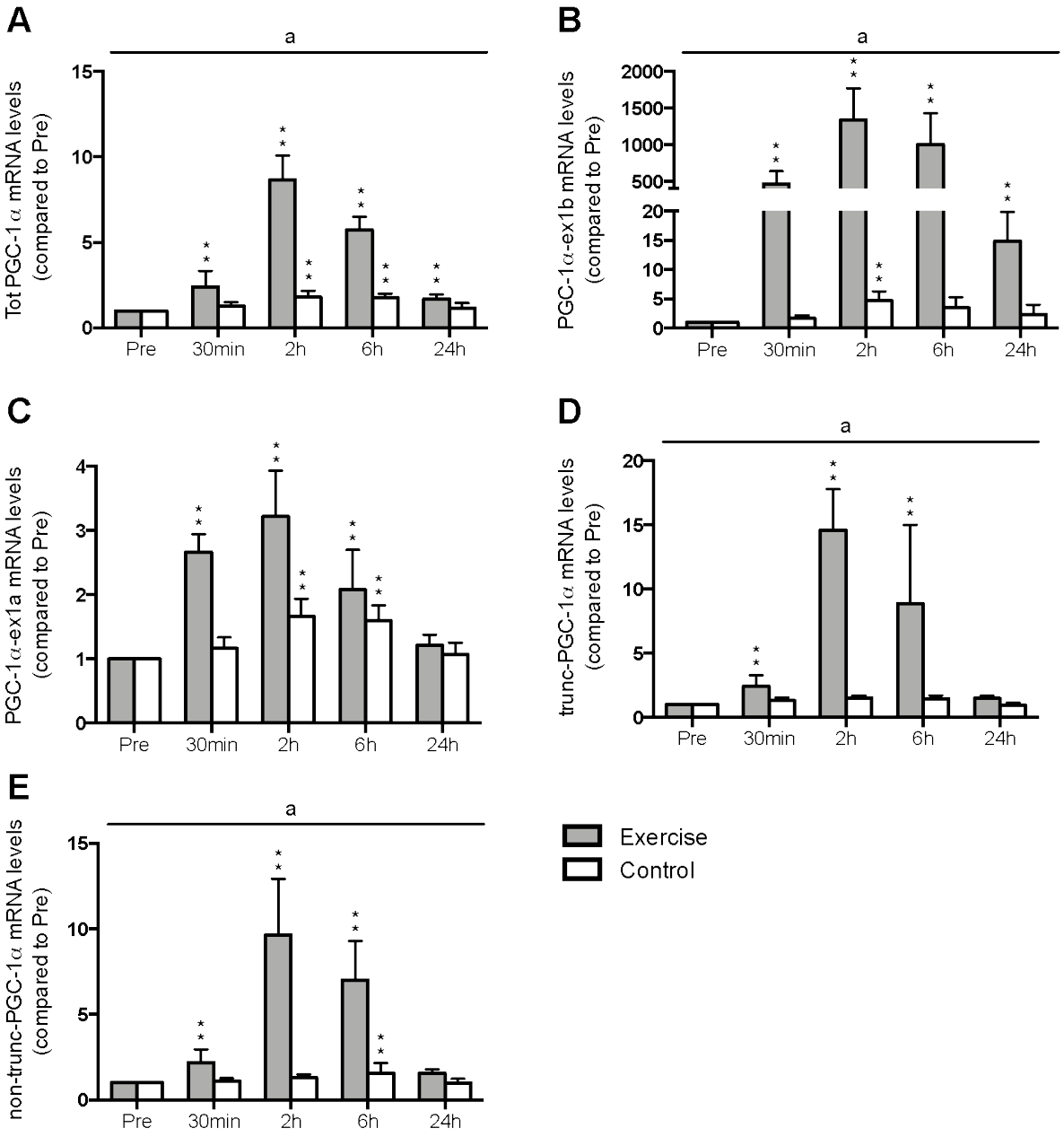

Figure 13. Skeletal muscle mRNA expression of PGC-1α variants after aerobic exercise. mRNA levels of (A) Total PGC-1α, (B) PGC-1α-ex1b, (C) PGC-1α-ex1a, (D) trunc-PGC-1α, and (E) non-trunc-PGC-1α, in human skeletal muscle before and after (30 minutes, 2 hours, 6 hours, and 24 hours) one bout of aerobic exercise (n = 12) or in non-exercising control (n = 5). $^{a}P \leq 0.05$ with factors time × group, $^{**}P \leq 0.01$ compared to Pre. Values are presented as mean ± SEM.

In Paper I, a comparison of the truncated and non-truncated transcripts and the expression from exon 1a and -b, respectively, before and after one bout of aerobic- and resistance

exercise was made. This was done to further elucidate if aerobic- or resistance exercise induce expression of PGC-1α from a specific exon, or induce the truncated variants specifically. There were no significant differences between the two exercise groups (Figure 14). However, one bout of exercise, irrespective of modality, changed the distribution between transcripts originating from exon 1a and -b; the PGC-1α-ex1b expression increase was much greater than the PGC-1α-ex1a increase.

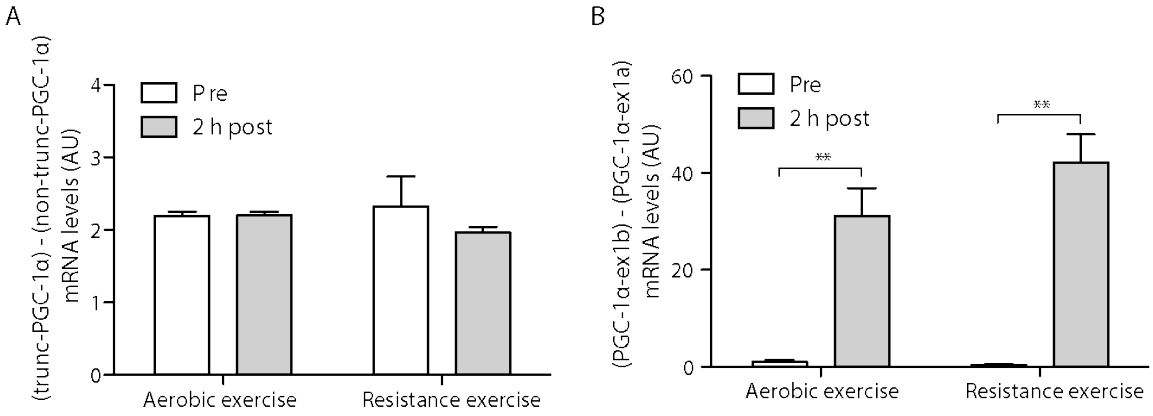

Figure 14. Comparison of PGC-1α mRNA expression from exon 1b and -a and the truncated and non-truncated variants in response to aerobic or resistance exercise. Comparison of the mRNA expression (A) of the truncated to the nontruncated PGC-1α variants (trunc-PGC-1α – non-trunc-PGC-1α) and (B) from exon 1b and exon 1a (PGC-1α-ex1b – PGC-1α-ex1a) before (pre) and two hours after exercise. Values are expressed as mean ± SEM. **Represents $P < 0.01$ pre *vs.* 2 h post.

To further test if PGC-1α4 regulates muscle hypertrophy specifically, cultured human myotubes were treated with AICAR as a metabolic stimulus (AMPK stimulator, Paper I). Activation of the AMPK pathway resulted in a significantly higher expression of both truncated and non-truncated PGC-1α variants (Figure 15). Also, although not statistically significant ($P = 0.07$), the truncated variant was induced almost twice as much numerically as the nontruncated variant. Also, other studies propose that the expression from ex1b are less sensitive to AMPK activation and expression from ex1b are more sensitive to β-adrenergic stimulation combined with AMPK activity (Norrbom et al. 2011; Miura et al. 2008; Popov et al. 2017).

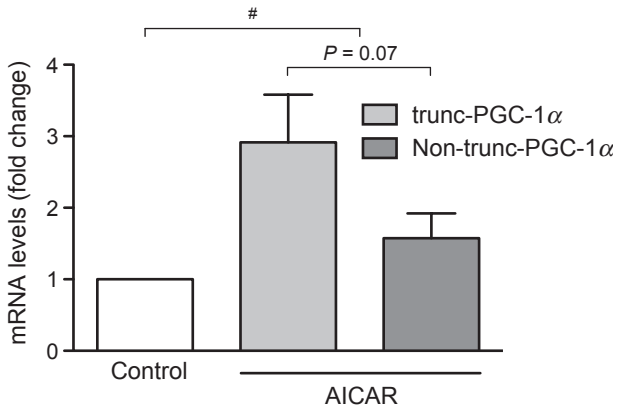

Figure 15. mRNA expression of PGC-1α variants in AICAR-stimulated human myotubes. mRNA expression in human myotubes of trunc-PGC-1α and non-trunc-PGC-1α after stimulation with AICAR for 24 hours compared to unstimulated control myotubes. $n = 4$. $^{\#}P < 0.05$, interaction between expression of trunc-PGC-1α and non-trunc-PGC-1α. Values are presented as means ± SE.

Collectively, these findings do not support PGC-1α4 as specifically regulating muscle hypertrophy. The control of both hypertrophy and aerobic adaptations are most likely coordinated through a much broader array of transcription regulators.

4.6 TRANSCRIPTIONAL REPRESSORS AFTER ACUTE AEROBIC EXERCISE

To further investigate the regulatory network of transcription after exercise and exercise training transcriptional corepressors were measured (Paper II). The mRNA expression of the transcriptional corepressor RIP140 was not significantly induced by aerobic exercise compared to the non-exercising control (P=0.06, Figure 16A). However, when analysing the exercise group only, the RIP140 mRNA was significantly increased during the whole recovery period up to 24 hours after exercise. RIP140 protein levels were rather inconclusive, however significantly different over time in the exercise group compared to the control group with increases in both groups at different time points (Figure 16B).

To further study RIP140 protein after exercise, nuclear- and cytoplasmic fractions were analysed and showed that the protein is only present in the nuclear fraction (Figure 16C). This was not surprising since RIP140 acts as a transcriptional corepressor. There were no significant changes in RIP140 nuclear levels which indicates that this protein in not translocated upon possible activation, rather it is always present in the nucleus.

Previous studies have shown induction of RIP140 by exercise in humans (Frier et al. 2011; Edgett et al. 2013). Hence, RIP140 is probably important for the coordination of the complex transcription regulatory network after acute exercise.

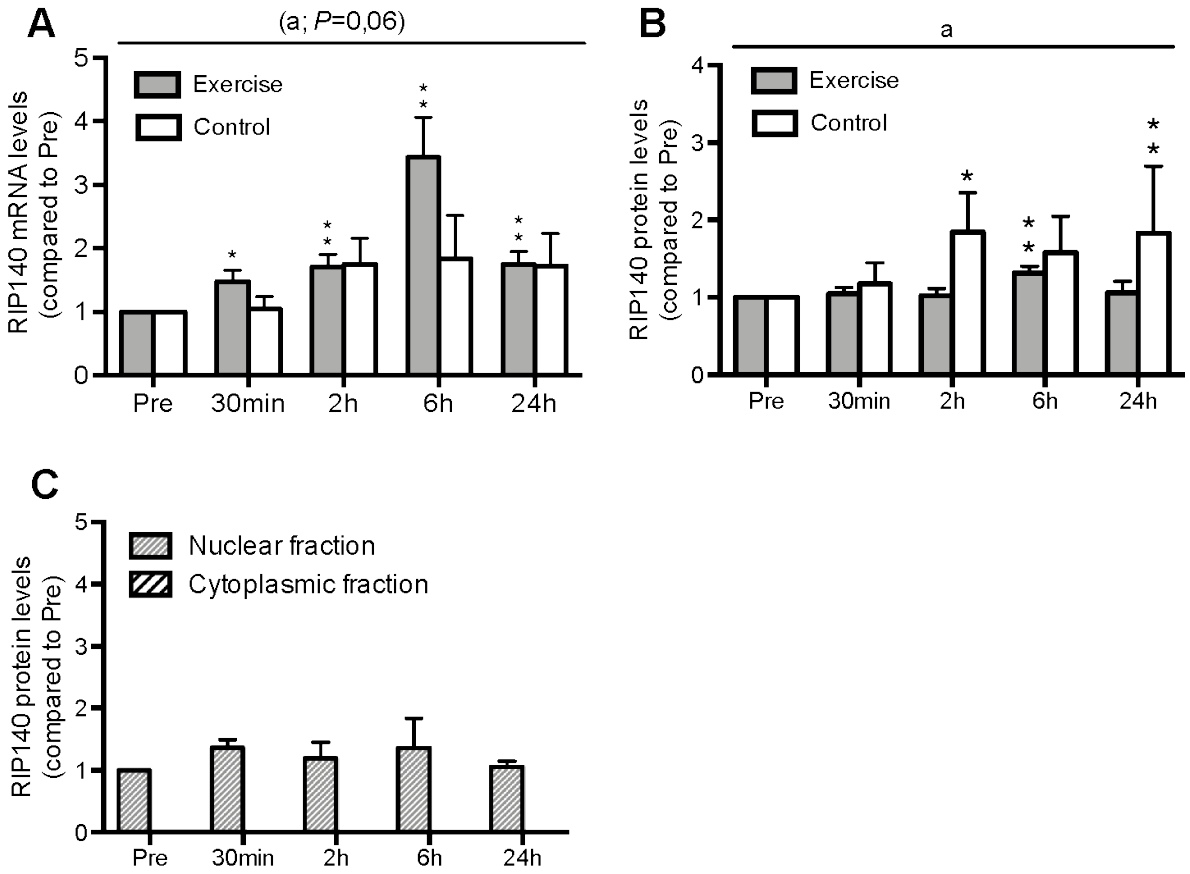

Figure 16. Skeletal muscle RIP140 mRNA and protein after acute aerobic exercise. Levels of (A) RIP140 mRNA, (B) RIP140 protein, and (C) nuclear and cytoplasmic RIP140 protein, in human skeletal muscle before and after (30 minutes, 2 hours, 6 hours, and 24 hours) one bout of aerobic exercise or in non-exercising control. n=15 and n=5 in the exercise- and control group, respectively. $^{a}P \leq 0.05$ with factors time × group, $*P < 0.05$ compared to Pre, $**P \leq 0.01$ compared to Pre. Values are presented as mean ± SEM.

Apart from RIP140, the expression levels of the transcriptional repressors NCoR1, p107, and Rb were measured. Rb steady-state mRNA- and protein levels were unaffected by three weeks of HIIT (Paper III; Figure 1A and 2A, respectively). NCoR1 mRNA levels after acute aerobic exercise were not significantly different from the control group (Paper II; Figure 6B-E). However, when the two groups were analysed separately there were differences at the various biopsy time points, although inconclusive since there was not a clear pattern of induction/repression of transcription. However, three alternative transcripts were detected, but further studies are needed to elucidate what role they may have in skeletal muscle adaptation to exercise and exercise training (Paper II; Figure 1).

Steady-state mRNA levels of p107 (Paper III) were unaffected by three weeks of HIIT (Figure 17A). However, p107 protein levels were reduced with more than half compared to

before training, probably leading to de-repression of gene expression of its target genes (Figure 17B).

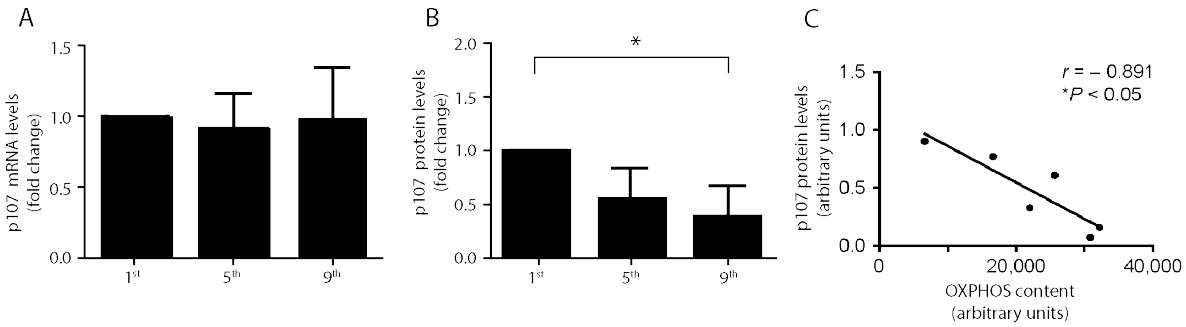

Figure 17. Skeletal muscle p107 mRNA and protein after acute exercise, and correlation with OXPHOS content, during and after three weeks of HIIT. Levels of (A) p107 mRNA and (B) p107 protein in human skeletal muscle at steady-state before the 1st (baseline), 5th, and 9th exercise bout, n=11, $*P \leq 0.05$ compared with baseline. Values are presented as means ± SD. (C) Correlation between p107 and total OXPHOS protein levels at steady-state after three weeks of HIIT, n=6.

To test the potential involvement of p107 in regulating genes important for oxidative metabolism correlations between p107 and OXPHOS protein content (data from (Ydfors et al. 2016)) after exercise training were done. It was evident that there was an inverse association of steady-state p107 and OXPHOS protein content after three weeks of HIIT, both for all complexes in total (Figure 17C) and for each complex alone (Paper III, Figure 5).

4.7 DOWNSTREAM TARGETS OF PGC-1α AFTER AEROBIC EXERCISE

PGC-1α affect the expression of many factors important for skeletal muscle remodelling. Confirmative of previous studies (Breen et al. 1996; Gustafsson 2011), the levels of VEGF-A mRNA were significantly increased by an acute bout of aerobic exercise, with a peak induction during the first 2-6 hours of recovery and back to baseline levels after 24-hours (Figure 18A). The truncated forms of PGC-1α (PGC-1α4 and NT-PGC-1α) is shown to strongly induce VEGF expression while having only a minor effect on mitochondrial genes (Thom et al. 2014). Accordingly, the early induction of PGC-1α-ex1b protein may partly explain the early increase in VEGF-A expression. However, VEGF-B were unaffected (Figure 18B). This confirms a previous study where exercise failed to induce VEGF-B (Kivelä et al. 2007) and another study concluding that VEGF-B is dispensable for angiogenesis but crucial for blood vessel survival (F. Zhang et al. 2009). Hence, to further study VEGF-B after exercise training may clarify its potential role in exercise mediated angiogenesis.

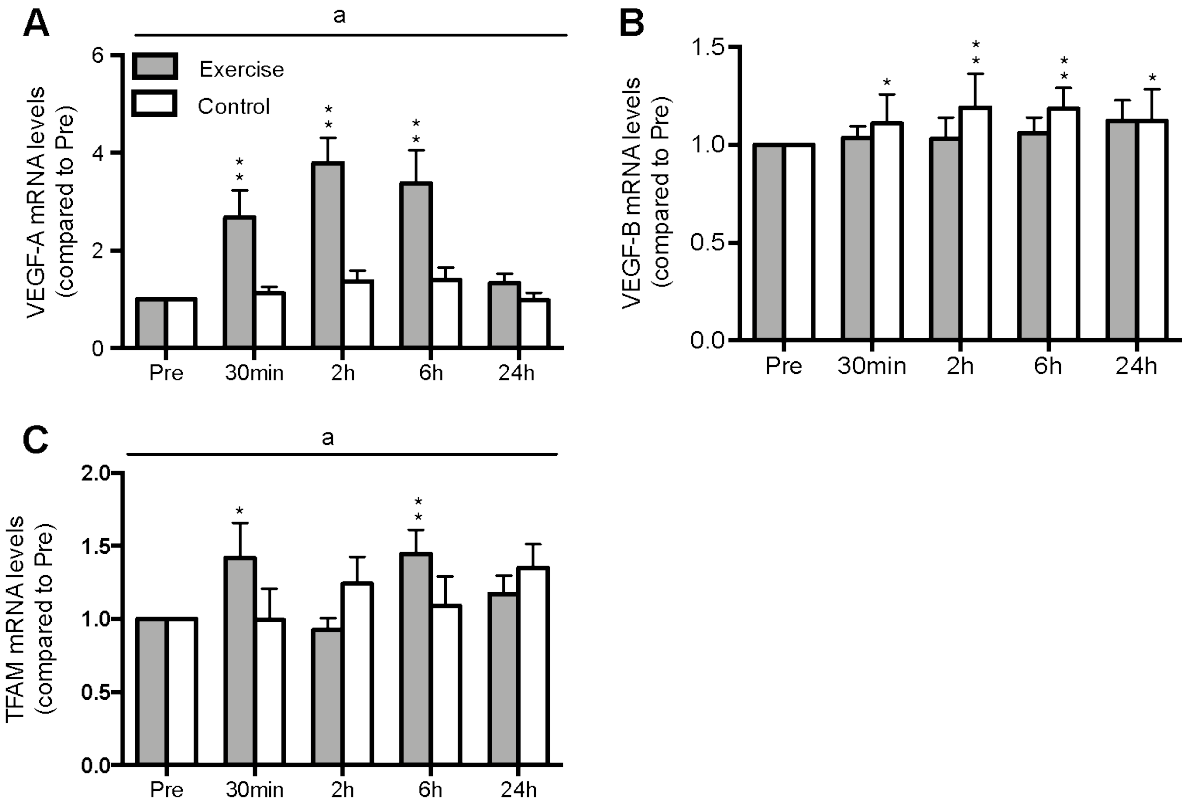

Figure 18. Skeletal muscle mRNA expression of downstream targets of PGC-1α after acute aerobic exercise. Levels of (A) VEGF-A, (B) VEGF-B, and (C) TFAM mRNA, in human skeletal muscle before and after (30 minutes, 2 hours, 6 hours, and 24 hours) one bout of aerobic exercise (n=15) or in non-exercising control (n=5). $^{a}P \leq 0.05$ with factors time × group, $*P < 0.05$ compared to Pre, $**P \leq 0.01$ compared to Pre. Values are presented as mean ± SEM.

The mRNA expression of the PGC-1α downstream target TFAM were significantly different between the exercise- and control group (Figure 18C). However, the increase seen in the exercise group was relatively small and inconclusive with a significant increase 30 minutes and six hours after exercise but not at the biopsy time point in between (2 hours). TFAM protein levels have previously been shown to be higher in endurance trained elite athletes compared to moderately active individuals (Norrbom et al. 2010). This may show its involvement in the maintenance of oxidative capacity, or only reflects the higher mitochondrial mass in well-trained individuals. TFB1M and SDH subunit A were both unaffected by acute exercise (Paper II, Figure 5). TFB1M has been shown previously to be increased by exercise training (Norrbom et al. 2010). Hence, all downstream targets of PGC-1α, p53, and other key regulators of exercise adaptation, needs to be further studied during skeletal muscle adaptation as well as in a more 'trained' state.

5 CONCLUSIONS

With three weeks of high-intensity interval training there was an overall attenuation of the transcriptional response to a single exercise bout in human skeletal muscle. However, the response pattern was strikingly reproducible and independent of training status since the vast majority of differentially expressed genes at the last exercise bout were also regulated at the first bout, albeit to a lesser degree. This finding provides comprehensive evidence for the very fundamental concept that 'trained' skeletal muscle experiences less stimulus, *i.e.* less change in its internal and external environment, because it is able to maintain cellular homeostasis during acute exercise better than 'untrained' muscle does. Additionally, there were different genes and pathways responding to acute exercise compared to regular exercise training over three weeks. Thus, generally speaking, specific pathways seem to orchestrate skeletal muscle remodelling following acute exercise while others seem to coordinate the maintenance of human skeletal muscle adaptation.

At least four variants of PGC-1α were found to be present in human skeletal muscle, and all were increased by both aerobic- and resistance exercise. Thus, there was no support for exercise-modality specific variants and all variants might be important, perhaps to different degrees, during the recovery period irrespective of exercise modality. It seems as if skeletal muscle adaptation to exercise is far more complex than to ascribe one, or even a few, transcriptional regulators any dominant role. Accordingly, the control of both hypertrophy and aerobic adaptations are most likely coordinated through a much broader array of transcription factors, and other molecular mechanisms.

This thesis shows that the various PGC-1α variants may play different roles at different phases in the transcriptional regulation of skeletal muscle remodelling. Since PGC-1α-ex1b protein is induced earlier than PGC-1α protein after acute aerobic exercise it is likely that PGC-1α-ex1b plays a more important role in the very early phase of gene expression modulation. To date, more emphasis has been put on transcriptional activators than on transcriptional repressors in studies of human skeletal muscle adaptation. The transcriptional repressors RIP140 and p107 were affected by acute exercise and exercise training, respectively, which highlights the possible importance of transcriptional repressors in the regulation of skeletal muscle adaptation to exercise training in humans.

This thesis also stresses the importance of including a non-exercising control group in exercise intervention studies. The inclusion of a control-group minimises the risk of

assigning the exercise stimulus as the sole inducer of mRNA and protein changes following exercise. Other factors, *e.g.* inflammation due to the biopsy procedure or diurnal fluctuations, could affects the parameters measured following exercise.

6 FUTURE PERSPECTIVE

Ranging from a basic scientific perspective to the benefits for patients, the general public, and the elite athlete, it is of great importance to create a basic understanding of the molecular events that precede the skeletal muscles' structural and functional improvements induced by exercise training. Exercise training is a powerful stimulus for improving cardiorespiratory fitness and skeletal muscle remodelling with subsequent improvements in health and performance. The findings in this thesis encourage future exercise studies with various types of training interventions and with participants with different training status.

To study the skeletal muscle molecular response in well-trained individuals would give more insight into the molecular network regulating the tissue remodelling as well as the maintenance of highly adapted muscle tissue. Further, cross-sectional comparisons of the global transcriptional response to acute exercise in well-trained individuals and sedentary controls would also add important information on this topic. Future studies should also focus on how individual pathways are activated by acute exercise during the adaptation process, and how exercise intensity relates to the activation of these pathways.

This thesis has shown that the global transcriptional response to acute exercise is blunted with training, providing comprehensive evidence for the very fundamental concept that 'trained' muscle experience less stimuli because it is able to maintain cellular homeostasis during acute exercise better than 'untrained' muscle do. However, proteomic studies, together with studies of protein-protein interactions and subcellular locations of transcription factors and -coregulators, as well as transcription factor binding to DNA, are also needed to broaden the mechanistic understanding of skeletal muscle remodelling. An additional aspect of future studies could be to explore how individual responsiveness to exercise training relates to the global molecular response. This could aid in the attempt to elucidate the variation in individual responsiveness to exercise training and potentially be beneficial for tailored individual training programs for patients and athletes.

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Våra kroppar är gjorda för rörelse. Fysisk aktivitet har många väldokumenterade positiva effekter för både hälsa och prestationsförmåga. Våra skelettmuskler har dessutom en enastående förmåga att anpassa sig till de ökade krav som ställs vid fysisk aktivitet. Ur såväl ett grundforskningsperspektiv, som ur nyttoperspektivet för patienter, allmänhet och elitidrottare, är det av stor vikt att öka kunskapen och förståelsen om de molekylära processer som föregår skelettmuskelns anpassning till träning.

Skelettmusklerna är till största del uppbyggda av avlånga muskelfibrer (muskelceller) organiserade i buntar, se Figur 1 på sida 9. Utöver muskelfibrer består muskeln av bland annat av satellitceller (en typ av stamceller), nerver, bindväv, fettceller och kapillärer. De minsta enheterna inuti muskelcellerna kallas för sarkomerer och det är dessa enheter som drar ihop muskeln (kontraherar). Sarkomererna består bland annat av två proteiner, aktin och myosin, som glider på varandra och skapar den mekaniska kraft som kontraherar muskeln. Denna process kräver energi i form av molekylen ATP. ATP bildas när framförallt kolhydrater eller fett bryts ner i närvaro av syre i strukturer i skelettmuskelcellen som kallas mitokondrier. Mitokondrierna brukar kallas för ’cellens kraftstationer’ just på grund av att de står för majoriteten av cellens ATP bildas. Som en del i anpassningen till uthållighetsträning bildas fler och större mitokondrier vilket leder till att muskeln blir bättre på att tillverka ATP med hjälp av syre och därmed blir mer uthållig.

Skelettmuskelns anpassning till träning, till exempel muskeltillväxt eller fler mitokondrier, initieras av de förändringar som sker i muskeln vid träning. Dessa förändringar är till exempel lägre energinivå (färre ATP molekyler), förändrat pH och ökade kalciumnivåer i skelettmuskelcellen. Förändringarna fungerar som signaler som initierar att generna i muskelcellkärnans DNA används. När en gen används bildas en kopia som kallas för mRNA, vilken i sin tur fungerar som en mall vid tillverkningen av ett specifikt protein. Dessa proteiner kan till exempel vara aktin och myosin som behövs för att tillverka fler sarkomerer som drar ihop muskeln, byggstenar för fler mitokondrier, enzymer som driver på kemiska reaktioner eller så kallade transkriptionsfaktorer. Transkriptionsfaktorer är reglerande proteiner som binder till DNA i cellkärnan och påverkar vilka gener som används och hur många mRNA-molekyler som tillverkas från varje gen.

Syftet med den här avhandlingen var att utöka kunskapen om de processer inuti muskelcellerna som är involverade i muskelns anpassning till träning. Detta gjordes genom

att ta muskelprover innan och efter träning på friska försökspersoner och mäta mängden mRNA och proteiner i skelettmusklerna efter ett pass av uthållighets- eller styrketräning, samt vid flertalet tillfällen under tre veckor av regelbunden högintensiv intervallträning.

Avhandlingen visar att mRNA för ett protein (PGC-1α) som visat sig vara central vid anpassningen till uthållighetsträning, finns i flera varianter i skelettmuskeln efter ett träningspass. Dock verkar ingen av dessa varianter vara specifik för uthållighets- eller styrketräning. Skelettmuskelns anpassning till träning är sannolikt mycket mer komplex än att ett, eller ett fåtal, proteiner reglerar till exempel muskeltillväxt eller uthållighet specifikt. Majoriteten av tidigare forskning på transkriptionsfaktorer och träning är fokuserad på de proteiner som ökar mRNA mängden i cellen. Den här avhandlingen visar att två mRNA/protein som minskar mängden mRNA (RIP140 och p107) var påverkade av träning vilket styrker att även negativ reglering är en viktig del i koordinationen av skelettmuskelns anpassning till träning.

Med hjälp av en studie där försökspersoner under tre veckor genomförde nio stycken högintensiva intervallträningspass kunde det visas att ökningen av mRNA-mängden efter det sista träningspasset var dämpat jämfört med efter det första passet. Detta visar på en grundläggande princip att när skelettmusklerna blir mer anpassade, alltså bättre tränade, får de inte lika starka signaler vid träning. Trots det dämpade svaret vid sista träningspasset var det slående hur pass likartat reaktionsmönstret var oberoende av träningsstatus. De mRNA som ökade vid första passet var till största del ökade även vid sista passet men då i lägre grad. Dessutom var det till stor del andra slags mRNA som ökade efter ett enstaka träningspass jämfört med efter tre veckors regelbunden träning. Detta tyder på att det är olika gener som reglerar de förändringar som sker i skelettmuskeln vid anpassning till träning än de som koordinerar upprätthållandet av den förbättrade muskeln.

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